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tion of functional residual air, with certain additions: (a) sets of evacuated gas sampling tubes are attached to the inspiratory tubing (at *A*), and to the expiratory tubing (at *B*); (b) a side tube leads off from a three-way valve, between inspiratory flutter valve and mouthpiece, for the collection of alveolar air samples; (c) a shut-off valve is interposed between expiratory tube and soda lime bottle. The volume of the dead space of this breathing circuit, as measured by Christie's method, was 1830 cc.

The method, briefly stated, was to fill the spirometer with a measured volume of oxygen, to have the subject breathe through the circuit until redistribution of inert gas (nitrogen) between lungs and spirometer was completed; then to take simultaneous samples of inspired and expired air, at successive intervals during this subsequent equilibrium period. Our interest lay in the nature of the equilibrium state existing after the initial redistribution of gases was completed.

The time required for redistribution of inert gases between lungs and spirometer in an apparatus of this kind has been estimated by Van Slyke and Binger (1) to be about 5 minutes. These investigators used a hydrogen-oxygen mixture in the spirometer, and found that at the end of 5 minutes of normal quiet respiration, the  $N_2/H_2$  ratio in the spirometer reached a level which thereafter remained essentially constant.

The details of technique in our experiments were as follows. With the subject (usually under basal conditions) attached to the apparatus by mouthpiece and noseclip, and breathing room air through Valve I, samples of alveolar air were first taken by having him make a single forced expiration through Valve III and the side tube, Valve I being turned from room air to apparatus. When respiratory equilibrium was again established (after 3 minutes), Valve I was turned, at the end of a normal *expiration*, and the subject then breathed for 8 minutes in the closed circuit. During this time oxygen was, of course, being absorbed from the system, and the oxygen concentration was therefore progressively decreasing. The course of respiration and of oxygen consumption was recorded on the drum.

At the end of 6 minutes' breathing, a sample of air was taken, during the course of an expiration, at *A*. This was the air which *was to be inhaled*

at the next following inspiration. The subject was then allowed to take this next inspiration. At the end of the immediately following expiration, an expiratory sample was taken at *B*. Thus, in the *A* and *B* samples, as taken, we had essentially the composition of an inspiratory sample of air, and of the last part of the next following expiration.

The *A* sample would, of course, represent the exact composition of all the inspired air of the next breath, only if the composition of air were constant along the whole inspiratory tube, from spirometer to mouthpiece, and if the volume of inspired air equalled the volume of this inspiratory tubing. The volume of inspiratory tubing was about 420 cc.; and as the spirometer air differed in  $O_2$  and  $N_2$  concentrations from air in *A* samples by only about 0.25 per cent, it is probable that the average value of nitrogen concentration of inspired air differed from the corresponding *A* sample nitrogen concentration by less than 0.1 per cent.

Similar *A* and *B* samples were obtained at the end of 7 minutes' breathing, and at the end of 8 minutes. Immediately upon taking the 8th minute expiratory sample, Valve III was turned, the subject directed to make a forced deep expiration, and an alveolar air sample was taken from the side tube.

The gas samples were analyzed by a Haldane apparatus, provided with a burette graduated between 5.0 and 10.0 cc.

Several experiments of this type were run on each of three normal individuals. The results of all were consistent.

The results of a typical experiment, with the normal subject A.C., are given in Table I. The first horizontal row of figures shows the concentrations of gases in the alveolar air sample taken while the subject breathed room air; the "*A* sample" here represents room air, the "*B* sample" alveolar air. It will be seen that expired nitrogen concentration is greater than inspired nitrogen concentration, as one would expect.

The next three horizontal rows of figures give the concentrations of gases in inspired (*A*) and expired (*B*) samples, at the end of 6, 7 and 8 minutes' breathing, in the closed circuit, during which time the oxygen concentration in the system was steadily decreasing, and nitrogen concen-

TABLE I

Composition of inspired (A) and expired (B) air, with progressively decreasing oxygen in inspired air (closed circuit), Subject A.C., December 3, 1935

Specimen	Ventilation	CO <sub>2</sub>		O <sub>2</sub>		R.Q.	N		ΔN <sub>2</sub>
		A	B	A	B		A	B	
	liters per minute	per cent	per cent	per cent	per cent		per cent	per cent	per cent
Alveolar, before.....		0.1	5.9	20.9	12.7	.72	79.1	81.4	-2.3
6th minute.....	4.63	0.0	5.5	48.6	43.7	1.10	51.4	50.8	+0.6
7th minute.....	4.85	0.1	5.6	46.2	41.3	1.12	53.7	53.1	+0.6
8th minute.....	4.82	0.1	5.4	43.3	39.0	1.23	56.5	55.6	+0.9
Alveolar, 8th minute.		0.1	6.2	43.3	37.6	1.06	56.5	56.2	+0.3
Spirometer, 8th minute.		0.1		43.0			56.9		

tration increasing. It will be seen that in these samples, expired nitrogen concentration is less than inspired nitrogen concentration. The differences are given in the last column on the right. Associated with this decrease in expired nitrogen concentration is a relative increase in expired oxygen concentration, so that the R.Q. as measured is greater than 1.

One's first thought in attempting to explain the excess of inspired over expired nitrogen, and the high R.Q., is that the subject was hyperventilating. From other data in the experiment, this

seems unlikely: as the expired CO<sub>2</sub> values were not low, were in fact near the alveolar CO<sub>2</sub> levels; the alveolar CO<sub>2</sub> at the end of the breathing period was not lower than at the beginning; and the total volume of ventilation was low. This question was, however, decided by experiment. Two further sets of breathing experiments were run, on each of two normal subjects, using the same apparatus: one set in which the inspired air was kept constant in composition; and the other in which the inspired air was increasing in its oxygen concentration during the 8 minutes of breathing. The first was arranged by removing the spirometer bell, having the subject inspire room air through the inspiratory (A) tube, and collecting expired air as it issued from the expiratory tubing. The second was carried out by running oxygen into an opening in the expiratory tubing at a constant rate of 500 cc. per minute.

In both these two accessory experiments it was found that the nitrogen concentrations of the expired air samples were regularly greater than those of the inspired air.

Figure 2 shows the results of the three sets of experiments. In the first, with progressively decreasing inspired oxygen, expired nitrogen concentration is less than inspired nitrogen concen-

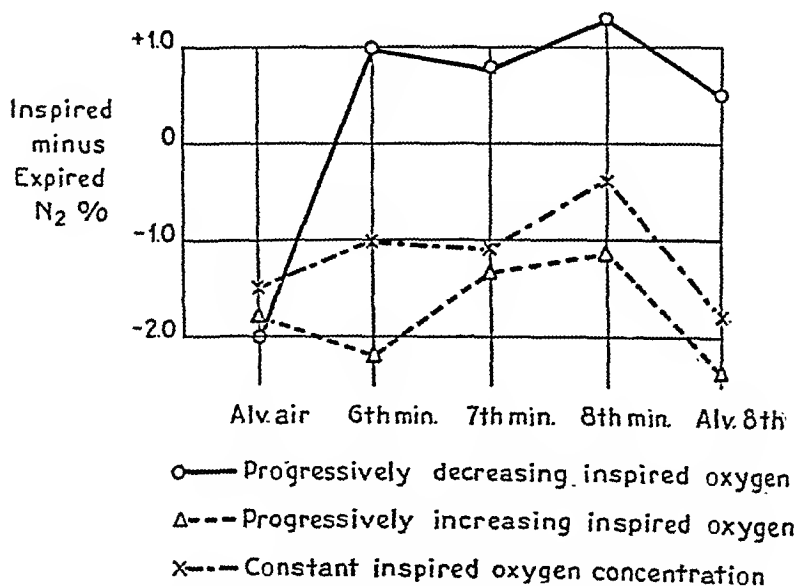


FIG. 2. DIFFERENCE BETWEEN INSPIRED AND EXPIRED AIR NITROGEN CONCENTRATIONS  $[\Delta N_2 (I-E)]$ .

Normal subject. For explanation see text.



tration; in the other two, with constant or increasing inspired oxygen, expired nitrogen is the greater. The alveolar air sample taken at the end of the 8th minute shows a similar effect, though in the decreasing oxygen experiment the nitrogen deficit is less marked. Some of this relative increase in expired nitrogen in the alveolar samples is due to the extra time (about 5 seconds) required for a complete forced expiration, with further concentration of nitrogen in the lungs in this interval.

The true explanation of this qualitative shift in nitrogen concentrations, in the decreasing oxygen system, is not difficult. If one has a volume of 1 liter of a gas mixture of 50 per cent oxygen and 50 per cent nitrogen, then adds to this liter a volume of 200 cc. of 45 per cent oxygen and 55 per cent nitrogen, the resulting concentrations of gases in the whole 1200 cc., after mixture, will be approximately 51 per cent nitrogen and 49 per cent oxygen (Figure 3). A mixed sample removed will, of course, have the same concentration.

In the same manner the lungs are a storehouse, as it were, of earlier breaths, all of which in this

instance have had higher oxygen and lower nitrogen concentrations than in the immediately preceding inspired sample.

Granting the above principle, one must next inquire whether in the actual breathing circuit which we have employed, the "oxygen storage effect" will be quantitatively sufficient to explain the experimentally found excess of inspired over expired nitrogen concentrations.

If we take figures from a group of experiments performed with normal subjects we find that the average change in composition of inspired air per breath is  $-0.25$  per cent for oxygen and  $+0.25$  per cent for nitrogen. Other average measurements, obtained experimentally, of lung volumes, ventilation, and composition of alveolar air, are as follows:

Functional residual air .....	2160	cc.
Tidal air (inspired) .....	495	cc.
Tidal air (expired) .....	490	cc.
Alveolar $\text{CO}_2$ .....	5.50	per cent
Respiratory dead space (approximate) .	100	cc.
Respiratory quotient .....	0.81	
$\text{CO}_2$ excreted per breath .....	21.5	cc.
$\text{O}_2$ absorbed per breath .....	26.5	cc.
Respiratory rate, per minute .....	10	

Using these figures we wish to calculate the gaseous composition of a series of expired breaths; as successive inspired breaths are inhaled, mixed with existing pulmonary air, and again exhaled; each inspired breath containing 0.25 per cent more  $\text{N}_2$  than its predecessor.

For convenience we will arbitrarily choose, as a starting point, a condition of the breathing circuit in which nitrogen is equal in lungs and spirometer. Suppose the concentration of nitrogen to be 50 per cent in spirometer, and 50 per cent in lungs, at the end of an inspiration; the alveolar  $\text{CO}_2$  5.50 per cent, and alveolar oxygen 44.50 per cent.

As expiration is carried out, 490 cc. are exhaled. The first 100 cc. (dead space) contain unchanged inspired air; the remaining 390 cc. contain:  $5.50 \text{ per cent} \times 390 = 21.5 \text{ cc. of } \text{CO}_2$ ;  $44.50 \text{ per cent} \times 390 = 173.5 \text{ cc. of } \text{O}_2$ ; and  $50.0 \text{ per cent} \times 390 = 195 \text{ cc. of } \text{N}_2$ .

There also remains in the pulmonary dead space 100 cc. of expired air of the above concentrations.

The next inspired air will contain 49.75 per cent  $\text{O}_2$ , and 50.25 per cent of  $\text{N}_2$ . The volume is 495 cc.; only 395 cc. will, however, reach the alveolar spaces, as 100 cc. remains in the pul-

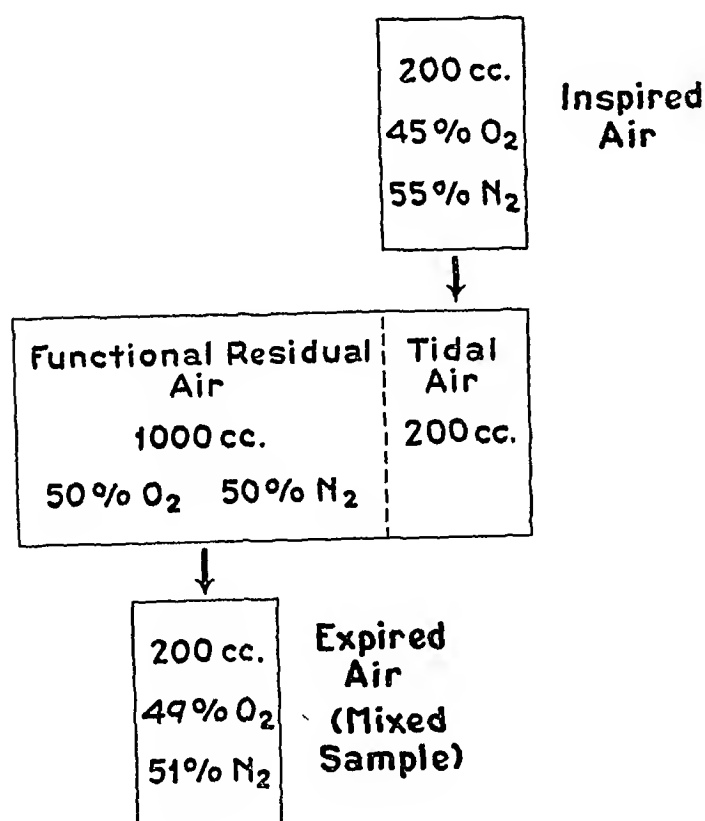


FIG. 3. DIAGRAM OF MIXING, WITH DECREASING INSPIRED OXYGEN.

# RESPIRATORY GAS IN CLOSED CIRCUIT. I

TABLE III  
Values of functional residual air, calculated (I) by assumption of equal mixture in breathing circuit, (II) by use of actual values of expired or alveolar air

Subject *	Date	Number of minutes breathing	I (Equal mixture)	II (Actual mixture)
H. C. A. L.	February 11, 1935	7	3660	3410
A. C. ....	January 24, 1935	7	2380	2210
A. C. ....	November 27, 1935	6	2477	2278
		7	2659	2367
A. C. ....	December 3, 1935	8	2890	2438
		6	2540	2312
		7	2560	2309
D. R. ....	January 3, 1936	8	2660	2343
		6	4300	3460
		7	4690	3518
		8	4945	3538

\* Total pulmonary volume of Subject A. C. was 6200 cc., vital capacity 4500 cc.; total pulmonary volume of Subject D. R. was 8100 cc.; vital capacity 5800 cc.

B, and alveolar air and spirometer values are obtained experimentally for the 8th minute, the necessary corrections for 6th and 7th minutes are easily made.<sup>2</sup>

It will be seen in Table III that the error due to assumption of equal mixture of nitrogen through the system is always appreciable, and may be considerable, in normal subjects. In Subject D. R., with large total capacity and large residual air, the excess in nitrogen concentration of inspired over expired air, after 6 to 8 minutes of breathing in the closed system, was about 1.3 per

cent, and the error due to the above assumption was therefore large.

## SUMMARY

1. When a normal individual breathes for several minutes in a small closed circuit in which the oxygen concentration is steadily decreasing, an equilibrium state is reached and maintained, in which the expired nitrogen concentration is less than the inspired nitrogen concentration. This is due to the progressive increase in inspired nitrogen concentration with each breath, to mixing of inspired air in the lungs with air previously inhaled, and to the exhalation of mixed air volumes by quiet breathing, in a closed circuit apparatus, use of alveolar air samples, obtained before and at the end of the breathing period, enables a correction to be made for the inequality of concentrations of inert gases through the system. This correction may amount to several hundred cubic centimeters in normal subjects.

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<sup>2</sup> The complete formula may be illustrated by the calculation of functional residual air for 7 minutes' breathing in the experiment of December 3, 1935 (see Tables I and III):

$$.814 V + (1830) (.791) + 65 = (.531 + .006) V + (1830 + 2090 - 60) (.537 + 0.004) + 60 (.511);$$

$V = 2190$  cc. (not corrected for temperature); where  
1830 = volume of dead space of apparatus,  
2090 = volume of excess oxygen in system,  
.531 + .006 = B sample nitrogen concentration corrected so as to equal alveolar nitrogen concentration at 7th minute,  
.537 + .004 = A sample nitrogen concentration corrected so as to equal spirometer nitrogen concentration at 7th minute,  
60 = volume removed from system when A and B samples are taken at end of 6th minute of breathing,  
(.60) (.511) = volume of nitrogen removed when A and B samples are taken at end of 6th minute of breathing.

changes this concentration from 39.0 to 38.1 per cent. Comparing these figures with the actual alveolar values of 6.2 per cent  $\text{CO}_2$  and 37.6 per cent  $\text{O}_2$ , one infers that the distribution of  $\text{CO}_2$  through the expirable part of the functional residual air is good, in this subject; the oxygen somewhat diminished in concentration in the deep alveolar specimen. There is, however, no large discrepancy; and mixture of gases through alveolar spaces can therefore be considered fairly satisfactory, so far as distribution of nitrogen is concerned.

The question remains whether the principles of mixture which we have just outlined are sufficient to explain the entire discrepancy between expired nitrogen values with decreasing oxygen in inspired air, as compared with these values with constant or increasing inspired oxygen. Our calculations indicate that quantitatively these principles are sufficient to explain this discrepancy. Whether there are also in the human lungs other methods of mixing or distributing successive inspired breaths, cannot be determined from present data.

The practical value of the above considerations is their application to the determination of residual air. In normal individuals the nitrogen concentrations of the alveolar air samples (corrected) can be taken to represent, approximately, the average nitrogen concentrations throughout the residual air. If determinations of alveolar air are made, therefore, both before and at the end of the period of breathing through the closed circuit, and the nitrogen concentration of the air in the spirometer determined as in the manner prescribed by the method of Christie (3), then the functional residual air can readily be calculated by a slight modification of Christie's formula.

The principle of the equation is the same: nitrogen in system at the start  $\pm 65$  cc. = nitrogen in system at the end of the breathing period.

In the above equation, also, we have altered Christie's practice slightly, by including the 65 cc. of nitrogen excreted from the lungs during the period of rebreathing; rather than making a flat correction in the final value of the residual air.

The equation in detail is as follows:

$$(V) (\text{Alv. } \bar{a}) + (\text{D.S.}) (.791) + 65 = (V) (\text{Alv. } \bar{p}) + (\text{D.S.} + \text{O}_2) (\text{Spirom. N}_2);$$

where

- $V$  = volume of functional residual air,  
 Alv.  $\bar{a}$  = alveolar nitrogen concentration taken while subject breathes room air,  
 Alv.  $\bar{p}$  = alveolar nitrogen concentration taken immediately after end of breathing through closed circuit,  
 D.S. = volume of dead space of apparatus,  
 $\text{O}_2$  = volume of excess oxygen in system at end of breathing period,  
 Spirom.  $\text{N}_2$  = concentration of nitrogen in spirometer at end of breathing period.

It is not actually necessary, for the purposes of this formula, to correct the alveolar samples for the number of seconds taken in obtaining them; inasmuch as the correction will be the same for each sample and will therefore cancel out as the equation is solved for  $V$ .

The above equation represents volumes at the temperature existing in the apparatus; after  $V$  has been calculated, the actual volume of air in the lungs is then obtained by correcting for the increase due to the temperature ( $37^\circ \text{C.}$ ) within the lungs; as has of course been described by Christie and others.

There is also a further small correction theoretically necessary due to the excess pressure of water vapor within the lungs (which must occupy some space). This pressure amounts to about 46 mm. in the lungs, and about 25 mm. in the apparatus at room temperature. The correction gives another slight increase to the figure for actual functional residual air; the ratio by which the calculated lung volume is multiplied being  $\frac{760 - 25}{760 - 46} = 1.02$ . This correction in normal subjects amounts to only about 50 cc. and may be neglected.

The correction provided by the inclusion of values of actual alveolar air (or expired air), in the calculation of functional residual air, is not negligible, as will be seen from Table III (first two experiments). As also shown in Table III, we have been able to calculate, by a few simple modifications of the formula just given, the values of functional residual air for each of the 6, 7 and 8 minute breathing periods, in the closed circuit experiments described in the first part of this paper, with decreasing inspired oxygen concentrations. This was accomplished by making small corrections in  $A$ - and  $B$ -sample values so as to make them approximately equal to spirometer and alveolar air values respectively. Since both  $A$  and

TABLE III

Values of functional residual air, calculated (I) by assumption of equal mixture in breathing circuit, (II) by use of actual values of expired or alveolar air

Subject *	Date	Number of minutes breathing	I (Equal mixture)	II (Actual mixture)
H. C. A. L.	February 11, 1935	7	3660	3410
A. C. ....	January 24, 1935	7	2380	2210
A. C. ....	November 27, 1935	6	2477	2278
		7	2659	2367
		8	2890	2438
A. C. ....	December 3, 1935	6	2540	2312
		7	2560	2309
		8	2660	2343
D. R. ....	January 3, 1936	6	4300	3460
		7	4690	3518
		8	4945	3538

\* Total pulmonary volume of Subject A. C. was 6200 cc., vital capacity 4500 cc.; total pulmonary volume of Subject D. R. was 8100 cc.; vital capacity 5800 cc.

B, and alveolar air and spirometer values are obtained experimentally for the 8th minute, the necessary corrections for 6th and 7th minutes are easily made.<sup>2</sup>

It will be seen in Table III that the error due to assumption of equal mixture of nitrogen through the system is always appreciable, and may be considerable, in normal subjects. In Subject D. R., with large total capacity and large residual air, the excess in nitrogen concentration of inspired over expired air, after 6 to 8 minutes of breathing in the closed system, was about 1.3 per

<sup>2</sup> The complete formula may be illustrated by the calculation of functional residual air for 7 minutes' breathing in the experiment of December 3, 1935 (see Tables I and III):

$$.814 V + (1830) (.791) + 65 = (.531 + .006) V + (1830 + 2090 - 60) (.537 + 0.004) + 60 (.511); \\ V = 2190 \text{ cc. (not corrected for temperature);}$$

where

- 1830 = volume of dead space of apparatus,  
 2090 = volume of excess oxygen in system,  
 $.531 + .006 = B$  sample nitrogen concentration corrected so as to equal alveolar nitrogen concentration at 7th minute,  
 $.537 + .004 = A$  sample nitrogen concentration corrected so as to equal spirometer nitrogen concentration at 7th minute,  
 60 = volume removed from system when A and B samples are taken at end of 6th minute of breathing,  
 $(60) (.511) =$  volume of nitrogen removed when A and B samples are taken at end of 6th minute of breathing.

cent, and the error due to the above assumption was therefore large.

## SUMMARY

1. When a normal individual breathes for several minutes in a small closed circuit in which the oxygen concentration is steadily decreasing, an equilibrium state is reached and maintained, in which the expired nitrogen concentration is less than the inspired nitrogen concentration. This is due to the progressive increase in inspired nitrogen concentration with each breath, to mixing of inspired air in the lungs with air previously inhaled, and to the exhalation of mixed samples.

2. In the determination of residual air volumes by quiet breathing, in a closed circuit apparatus, use of alveolar air samples, obtained before and at the end of the breathing period, enables a correction to be made for the inequality of concentrations of inert gases through the system. This correction may amount to several hundred cubic centimeters in normal subjects.

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# DISTRIBUTION OF RESPIRATORY GASES IN A CLOSED BREATHING CIRCUIT. II. PULMONARY FIBROSIS AND EMPHYSEMA

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In the preceding paper (1) we have shown that in a small closed breathing circuit of steadily diminishing volume (due to absorption of oxygen and  $\text{CO}_2$ ), any inert gas in the system will not be evenly distributed, when mixture of all respiratory gases reaches equilibrium. Due to the fact that the lungs are a reservoir containing air from many earlier breaths with correspondingly lower concentrations of nitrogen (or other foreign inert gas), the pulmonary air at any moment contains a lower nitrogen concentration than the spirometer.

It was found that with quiet breathing by normal subjects, tidal air was sufficiently well distributed through pulmonary spaces, so that by sampling either expired air or deep alveolar air, the concentration of nitrogen in the pulmonary air could be measured fairly accurately. By the use of this value as well as that of the sample from the spirometer, at the end of a period of from 6 to 8 minutes' quiet breathing, functional residual air could be calculated.

In the present investigation we have attempted to extend the same method of study to a group of cases of pulmonary fibrosis and emphysema.

It is clear that in the system under consideration, the lag in nitrogen concentration within the lungs, or difference between the nitrogen concentrations of inspired air and expired air, will depend upon a number of variables, among which are the volume of residual air in the lungs, the volume of effective tidal air (actual tidal air minus pulmonary dead space), the rate of change of inspired nitrogen (and oxygen) concentrations per breath.

Tidal air varied between 300 cc. and 600 cc., in the cases that we studied. Change in inspired nitrogen concentration per breath varied between 0.25 and 0.40 per cent.

If the functional residual air is small, one would

expect that there would be a more effective washing out of pulmonary air spaces with each breath, and less "oxygen storage" or "nitrogen lag" in the lungs during the closed circuit breathing.

We have made measurements of functional residual air in a group of cases of pulmonary fibrosis and other conditions tending to reduce residual air volume. The method used was that of Christie (2), modified by the additional use of alveolar air samples taken both before and at the end of the period of breathing in the closed circuit, as described in the preceding paper (1).

TABLE I

*Functional residual air, calculated (I) by assumption of equal mixture of nitrogen through system; (II) by actual measurement of expired (alveolar air) nitrogen.*

Subject	Age	Sex	Condition	Functional residual air	
				I	II
	<i>years</i>			<i>cc.</i>	<i>cc.</i>
H. L.	38	M	Normal	3660	3410
D. R.	40	M	Normal	4690	3518
A. C.	40	M	Normal	2380	2210
S. F.	25	M	Tuberculosis, fibrosis	2990	2570
G. L.	38	M	Tuberculosis, fibrosis	2405	2300
F. W.	28	F	Tuberculosis, fibrosis	1530	1400
A. M.	27	F	Tuberculosis, thoracoplasty	1168	1153
D. M.	50	M	Old empyema, fibrosis	1138	1128

Table I gives the residual air values for five such cases, and for three normal individuals. The principal pulmonary abnormalities of each case are listed. Each of these cases of pulmonary fibrosis was also studied quite extensively from the point of view of circulatory and pulmonary function. These details need not be reviewed here, but it is important to note that none of the cases had evidence of much disturbance in pulmonary gas exchange, or in distribution of tidal air through the lungs: that is,  $\text{CO}_2$  levels of alveolar air and of arterial blood were within normal limits, resting ventilation was not in-

creased (cf. Nielsen and Sonne (3)), and arterial oxygen saturation was normal at rest. (For technique of these studies see Cournand et al. (4)).

In Table I, the functional residual air has been calculated in two ways: first, assuming equal mixture of inert gas (nitrogen) through the system, and second by the use of actual pulmonary (alveolar) samples. It will be seen that the difference between the results by the two methods of calculation is less, the smaller the functional residual air. This is due largely to the fact (as predicted above) that with small functional residual air, concentration of nitrogen in expired air is not less than that in inspired, but is either the same or larger. In other words, the "nitrogen lag" in the lungs is much less than is the case with normal individuals having larger residual lung volumes. A given volume of tidal air washes out the existing residual air more effectively.

We may consider now the application of this method of study to cases of pulmonary emphysema.

Theoretically, as indicated above, a large residual air will tend to increase the "nitrogen lag" in the lungs, after the distribution of gases in the closed breathing circuit has reached equilibrium. It is possible to calculate what will be the expected nitrogen percentages in lungs and spirometer (or in inspired and expired air samples), after a given number of breaths, in a system with known values of residual air, tidal air, pulmonary dead space, and change per breath in nitrogen concentration of inspired air. As values typical of advanced emphysema we have chosen the following:

Functional residual air .....	5000	cc.
Tidal air (inspired) .....	403	cc.
Tidal air (expired) .....	400	cc.
Alveolar CO <sub>2</sub> .....	6.48	per cent
Pulmonary dead space .....	175	cc.
Respiratory quotient .....	0.81	
CO <sub>2</sub> excreted per breath .....	14.6	cc.
O <sub>2</sub> absorbed per breath .....	18.0	cc.
Respiratory rate, per minute .....	16	
Difference in inspired nitrogen concentration, per breath .....	0.25	per cent

The calculation is carried out in a manner similar to that described in the preceding paper. As one would expect, with larger residual air and smaller effective tidal air, it takes longer for an equilibrium state to be established, than was the

case with the normal values. It required, as a matter of fact, about 40 breaths before this equilibrium state existed, in the calculation with the above values. Table II shows the essential re-

TABLE II  
*Calculated changes in concentrations of respired gases, in closed circuit, with progressively decreasing inspired oxygen concentrations*

Assumed residual air	Number of breaths	Oxygen		Nitrogen		R.Q.
		In-spired	Ex-pired	In-spired	Ex-pired	
Normal 2160 cc.		per cent	per cent	per cent	per cent	
	1st breath	49.75	44.40	50.25	50.10	0.92
	10th breath	47.50	42.60	52.50	52.10	1.00
	12th breath	47.00	42.15	53.00	52.35	1.01
Emphysema 5000 cc.	1st breath	49.75	43.48	50.25	50.04	0.92
	15th breath	46.25	42.11	53.75	51.41	1.34
	20th breath	45.00	41.35	55.00	52.17	1.49
	30th breath	42.50	39.57	57.50	53.95	1.80
	40th breath	40.00	37.52	60.00	56.00	2.09

sults of this calculation, and compares them with those already given for normal values in Table II of the preceding paper (1). It will be noted how marked a discrepancy exists after 30 or 40 breaths, between inspired and expired air; resulting in an excess of 4 per cent in nitrogen concentration of inspired over expired air, and a respiratory quotient over 2.0.

It should be noted, in this calculation just as in that of the preceding paper, that we have made several assumptions here, especially that each inspired breath (exclusive of the pulmonary dead space) is evenly distributed throughout the functional residual air. Also, nitrogen excretion from the lungs has not been accounted for in our calculation; this would tend to increase expired nitrogen values slightly.

If, however, the conditions represented by the calculation are approximately fulfilled in emphysematous subjects, one would expect that the nitrogen concentration of expired air would be much less than inspired air, and that residual air calculated by the assumption of equal mixture of nitrogen through the system would have a much greater value than residual air calculated by the use of actual pulmonary nitrogen concentrations.

To test this postulate, we have made experiments, similar to those described in the preceding

paper, on several subjects with marked pulmonary emphysema. In these experiments, corresponding samples of inspired and of expired air were obtained, at the end of 6, 7, and 8 minutes' breathing in the closed circuit; and alveolar samples obtained before the closed circuit breathing began, and at the end of the eighth minute. The results of an experiment on each of two emphysematous subjects are given in Table III.

TABLE III

*Emphysema. Composition of inspired (A) and expired (B) air, with progressively decreasing oxygen concentration in inspired air*

Specimen	CO <sub>2</sub>		O <sub>2</sub>		RQ	N <sub>2</sub>		ΔN <sub>2</sub>
	A	B	A	B		A	B	
	per cent	per cent	per cent	per cent		per cent	per cent	per cent
SUBJECT S. B., FEBRUARY 18, 1936								
Alveolar, before.....	.03	6.2	20.9	12.3	0.72	79.1	81.5	-2.4
7th minute...	.02	5.1	26.6	22.1	1.08	73.4	72.8	+0.6
8th minute...	.06	5.3	22.9	18.3	1.15	77.0	76.4	+0.6
Alveolar, 8th minute.....	.06	7.2	22.9	15.9	1.03	77.0	77.0	0
SUBJECT F. S., FEBRUARY 19, 1935								
Alveolar, before.....	0	8.4	20.9	9.5	.74	79.1	82.1	-3.0
7th minute...	0	5.4	38.9	33.7	1.04	61.1	60.9	+0.2
8th minute...	0	6.0	36.1	31.1	1.20	63.8	62.9	+0.9
Alveolar, 8th minute.....	0	8.8	36.1	26.2	.89	63.8	65.0	-1.2

Both these subjects had advanced generalized pulmonary emphysema, with chronic bronchitis but without clinical evidence of any large localized pulmonary infection; nor any evidence of cardiac failure. The first patient, S. B., was a man of 38, whose symptoms were cough, and severe dyspnea, occurring always on slight exertion, and frequently in paroxysms even at rest. Physical examination showed characteristic signs of emphysema. His vital capacity was about 1,200 cc., his residual lung volume (according to our measurements) greatly increased. Arterial blood was 90 per cent saturated at rest. His symptoms were somewhat relieved by continuous oxygen therapy.

The second patient, F. S., was a man of 54 who had had chronic sinusitis for many years,

and for several years past, increasing cough and dyspnea on exertion. He was moderately cyanotic. Habitus was asthenic, with long chest, and atonic abdomen. Chest signs were characteristic of emphysema. The heart was not enlarged. Measurements of lung volume were variable, vital capacity varying from 1,500 cc. to 3,100 cc. (after adrenalin). Measurements of residual air were always high, though also variable. Intrapleural pressure was +10, -5. Arterial blood was markedly unsaturated, even at rest, varying between 78 and 88 per cent oxygen saturation. The patient was somewhat relieved by oxygen therapy, greatly relieved by adrenalin.

Examination of the figures in Table III shows, in the first place, in both subjects, a considerable discrepancy between the CO<sub>2</sub> and oxygen values of expired air as compared with corresponding values of alveolar air, at the end of the 8th minute. This discrepancy is greater than can be accounted for on the basis of the time taken by these patients (8 seconds by S. B., 10 seconds by F. S.) in making an extreme expiration, after a normal expiration. In the case of F. S., with the more marked emphysema, oxygen absorption in 10 seconds was about 50 cc., CO<sub>2</sub> output about 40 cc. Functional residual air, as measured, was 5,845 cc. Thus, the CO<sub>2</sub> content of the residual air, according to the expired air value, would be  $(5,845 \times .060) = 351$  cc.; whereas according to the alveolar air value it would be  $(5,845 \times .088) - 40 = 465$  cc. Correspondingly, the oxygen content of the residual air, according to the expired air value, would be  $(5,845 \times .311) = 1,818$  cc.; according to the alveolar air value, would be  $(5,845 \times .262) + 50 = 1,582$  cc.; a difference of 236 cc. The difference in nitrogen concentration of expired as compared with alveolar samples (Table III) is a further index of inequality of deep alveolar as compared with the last part of the expired tidal air.

Thus we have in the emphysematous subject evidence of considerable inequality of concentrations of respiratory gases through the lungs. This concept is not new. The most conclusive recent investigations have been those of Sonne and his collaborators (3, 5), who have shown that even in normal individuals there are alveolar spaces which are hypoventilated; whereas in emphysematous subjects this tendency becomes



very much more pronounced. In normal subjects there are apparently certain alveolar spaces which, though hypoventilated are also hypoperfused, so that the air in them is not greatly vitiated,  $\text{CO}_2$  being less in this air, and oxygen greater, than in other alveolar spaces. In patients with emphysema, on the other hand, all deeper alveolar air is high in  $\text{CO}_2$  and very low in oxygen, suggesting that the poorly ventilated regions are still relatively over-perfused by pulmonary blood.

It has been recognized for some years that the Haldane-Priestley "alveolar" specimen is a more or less empirical function; as evidenced by the fact that the correspondence in  $\text{CO}_2$  tension between alveolar sample and arterial blood depends upon the particular technique of obtaining this sample; and that this technique is different for different physiological conditions, such as rest and exercise. With Sonne's concept of the distribution of gases within the lungs, the Haldane-Priestley sample becomes frankly a roughly approximate mixture of air from various regions of the lungs.

In the case of the patient F. S., several determinations of the deepest possible alveolar air were found to be approximately the same in  $\text{CO}_2$  tensions, as the  $\text{CO}_2$  tension of arterial blood drawn at the same time. This suggests that there are regions in the lungs in which the air is more vitiated than that in the alveolar specimen; as well as hyperventilated regions in which the air is less vitiated.

Returning to the inspired and expired air values in Table III, we find that they are consistent with the concept just discussed. That is, the concentration of nitrogen in the expired air is not 3 or 4 per cent less than that in the inspired air, as one would expect from our calculations, but is less than one per cent less. This suggests that the volume into which the tidal air passes and in which it mixes with the air of earlier inspired breaths is about the same as the residual air volume of normal individuals (see data in preceding paper); rather than the much larger volume of total residual air in this emphysematous subject. The above suggestion is of course not to be construed strictly anatomically. Instead of a certain volume into which all tidal air is well and evenly distributed, there probably exists a certain reduced volume in which this even distribution

takes place, and in addition a large volume which some tidal air reaches but only in very small amount at each breath, so that such spaces contain at all times greatly vitiated air. The greater nitrogen concentration that always exists in deep alveolar specimens, as compared with that in the corresponding specimens of expired air, is consistent with this notion.

In a clinical case of emphysema there is apt to be much variation, from day to day, or even from hour to hour, in the severity of symptoms; and one would naturally expect corresponding variability in distribution of air through pulmonary spaces; associated with changes in patency of air passages, and in other functions. In a number of experiments, of the type illustrated in Table III, on a group of five emphysematous patients, our results quantitatively with each patient were quite variable. Expired air nitrogen was sometimes considerably less than inspired air, at another time nearly equal, or even greater. The values of alveolar air gases, and the relations between these and the expired air gases, or between these and outside air, were also variable.

Similarly, our efforts to obtain consistent results in values for functional residual air in such subjects, even after applying the correction for actual nitrogen concentrations of alveolar air (see previous paper), were unsuccessful. Some of these variations may have been due to actual change, from day to day, in resting mid-position of the chest; but frequently the variation in figure for functional residual air was considerably greater than could be explained by this factor, or by any probable alteration in pulmonary vascular bed or other anatomical cause.

Table IV gives some values obtained on three emphysematous subjects. Calculations were made as described in the preceding paper. It will be seen that several types of inconsistency exist in these residual air figures. In all subjects there is usually a large correction factor due to the use of actual alveolar air nitrogen values. This is due in part to "oxygen storage" and "nitrogen lag," in part to the fact that with a large amount of nitrogen in the system, dilution with a given volume of oxygen (run into the spirometer before rebreathing begins) causes less percentile change in total nitrogen concentration. The latter factor makes the divisor in the equation small, i.e., nitro-

TABLE IV

Figures for functional residual air in emphysematous subjects

Subject	Age	Sex	Date	Number of minutes breathing	Functional residual air	
					I (assuming equal mixture)	II (using alveolar samples)
A. G...	39	M	1935 March 24 March 27	7	cc. 3280	cc. 3200
				6	4160	3645
				7	4215	3640
				8	4410	3710
E. P...	56	M	January 13 January 16	7	6940	5930
				6	3560	2940
				7	3890	2965
S. B...	42	M	February 18 March 2 March 8 March 13	7	9150	5520
				6	5420	4650
				7	6550	5150
				8	8960	6750
				6	9390	6730

gen concentration in the lungs at the start minus nitrogen concentration at the end. A 2 per cent error in nitrogen concentration (due to failure to use actual alveolar nitrogen concentration) therefore produces a greater proportional effect upon this divisor, than if the divisor were numerically larger.

In Subject A. G. it will be seen that the corrected values for functional residual air, are fairly consistent. In Subject E. P., on the other hand, there occurred in two successive examinations a large and unaccountable difference in residual air value. This subject was particularly cooperative, and technically both tests appeared satisfactory. In Subject S. B. there was not only a very large difference between corrected and uncorrected values, but the former varied widely among themselves.

With Patient F. S., also, we tried for several weeks during the spring of 1934, and again at frequent intervals in the winter and spring of 1935, to establish a consistent level for functional residual air. Table V shows the results of a group of experiments. These were technically satisfactory, yet gave most diverse figures.

It will be noted that several of the experiments recorded in Table V were performed while the patient was in an oxygen chamber, in which the oxygen was maintained at 35 to 50 per cent. The effect of high oxygen upon anoxic subjects is

TABLE V

Measurements of functional residual air, under various conditions, in emphysematous subject (F. S.): I, assuming equal mixture in system; II, using actual alveolar nitrogen values

Date	Conditions	Vital capacity	Alveolar nitrogen	Functional residual air	
				I (equal mixture)	II (actual mixture)
		cc.	per cent	cc.	cc.
December 13, 1934	Room air	1810	81.4	5,150	4790
February 20, 1935	Room air	1785	81.7	6,055	5845
March 5, 1935	Oxygen room	2120	53.9	9,770	6440
March 11, 1935	Oxygen room, before adrenalin	1530	67.2	11,400	7730
March 11, 1935	45 minutes after 1 mgm. adrenalin	3100	70.7	3,370	2510
March 18, 1935	Oxygen room, before adrenalin	—	58.4	7,520	6400
March 18, 1935	45 minutes after 1 mgm. adrenalin	1710	60.6	5,160	3990

to increase greatly the alveolar and blood CO<sub>2</sub> levels, as well as to provide increased arterial oxygen saturation (Richards and Barach (6)). This probably occurs secondary to diminished ventilation of alveolar spaces. Thus, in Patient F. S., the alveolar CO<sub>2</sub> increased from about 8 per cent to about 11 per cent. So far as calculations of residual air are concerned this effect should increase the alveolar nitrogen concentration as compared with that of the inspired air; and this in turn will increase the correction factor provided by our formula (see preceding paper) for residual air, a formula which includes alveolar values in the calculation. Table V demonstrates this. Another factor acting in this same direction is the fact that the divisor in the corrected formula (i.e., the difference between nitrogen of alveolar air before and after the closed circuit breathing) is usually smaller when room air nitrogen is only 60 per cent instead of 79.1 per cent.

Even more striking are the effects of adrenalin, in this patient, upon the residual air figures, as shown in Table V; much greater than the corresponding changes in vital capacity. The mechanism of this is not entirely clear. Presumably, some of the effect is due to more adequate ventilation of deep hypoventilated alveolar spaces; partly because of greater patency of air passages, partly because of the general hyperventilation induced by adrenalin.

It should be emphasized that the wide discrepancies in these various figures for functional residual air occurred in cases of extreme emphysema. A group of less advanced cases might well have shown more consistent results with the method used.

In conclusion, it seems clear that the unequal distribution of respiratory gases, which has been shown to exist in the lungs of emphysematous subjects, makes it difficult, if not impossible, to obtain reliable measurements of residual air with the method which we have used, of quiet breathing in a small closed circuit. Most of the errors appear to be in the direction of producing figures for residual air that are too large.

We have been experimenting recently with a modified method, which includes a preliminary period of breathing pure oxygen, for the purpose of washing out the nitrogen of hypoventilated alveolar spaces; followed by a period of closed-circuit breathing in which the volume of the system is kept constant, and in which the diluent inert gas is largely helium (with greater diffusing velocity). These experiments are not yet sufficiently advanced to be reported.

#### SUMMARY

1. Subjects with small residual lung volumes have correspondingly small "nitrogen lag" within the lungs, after breathing through a small closed circuit whose total volume progressively diminishes. By "nitrogen lag" is meant the excess of concentration of nitrogen in inspired over that in expired air.

2. Subjects with large residual lung volumes (emphysema) would be expected to have large "nitrogen lag." Due, however, to poor distribution of tidal air through the lungs, this does not occur.

3. In emphysematous subjects, poor distribution of tidal air, with hypoventilation throughout a large part of the pulmonary air spaces, may produce large errors in the determination of residual lung volumes by methods of quiet breathing in a closed circuit.

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# COMPLEMENT FIXATION TESTS IN PERTUSSIS

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Bordet and Gengou (1), in 1906, performed serological tests on pertussis employing the organisms which they discovered and claimed to be the causative agent of whooping cough as the antigen for the complement fixation test. They got a positive reaction in most cases of the disease. Yet, later investigations failed to confirm these results.

Among those who confirmed their results were Renaux (2) who examined 73 cases, Chievietz and Meyer (3) with 112 cases and Giese (4) with 123 cases. In recent years good results were reported particularly by German authors who employed a method which will be described later. Keller, Klopstock and Klopstock (5) examined 131 sera of 88 cases with pertussis; 77 sera showed a positive reaction, 17 were doubtful, and 37 sera were negative. Gundel and Schlüter (6, 7) reported a study of 70 pertussis patients; 54 of these were positive, 3 doubtful, and 13 negative. In a later series of cases they found that of 140 sera from children with pertussis and suspicious cases, 105 gave a positive reaction. The reason given to explain the negative reactions were, either that the children did not suffer from whooping cough or were too young or too sick to form antibodies.

The method employed by the German authors mentioned above consists in preparing an antigen by using whole pertussis bacilli suspended in alcohol to which a small amount of lecithin is added. The addition of lecithin tends to render the result more specific and at the same time to decrease the anticomplementary effect of the antigen. In a recently published paper, Hansing (8), using the same method on the sera of 189 patients, found that 48 per cent were positive, 22 per cent doubtful, and 29 per cent negative.

The failure of many workers to obtain good results with complement fixation in pertussis may be explained by the fact that no method was available

to differentiate between pertussis and influenza bacilli. Only recently Povitzki (9) found that Bordet-Gengou bacilli grew well on a definitely acid medium (pH 5.0) whereas the Pfeiffer bacillus was inhibited by it. Furthermore, Gundel and Schlüter (10) found that genuine pertussis bacilli when injected intradermally into white rabbits always produced severe necrosis while influenza bacilli only caused infiltration and erythema.

Because, in the past, there was uncertainty regarding the differentiation of both organisms the proper antigen was not always employed for the complement fixation test. The agglutination method, on the other hand, did not prove successful owing to the insufficient amount of antibodies during the natural disease. Besides, spontaneous agglutination is often observed and nonspecific results thus obtained. If it could be shown that the complement fixation test becomes positive in pertussis and also follows the injections of bacillus pertussis, this would seem to be another point in favor of the Bordet-Gengou bacillus as the etiological factor in whooping cough.<sup>1</sup>

The problems studied were the specificity of the reaction, its value in the diagnosis of whooping cough, its occurrence during the disease and the duration of the antibody content.

## METHOD

The complement fixation test was carried out in the manner employed by Dr. Thomson of this laboratory for

<sup>1</sup> MacDonald and MacDonald (17) produced experimental whooping cough in two humans and protected two others by prophylactic injections of Sauer's vaccine. All four children developed a positive complement fixation test while two non-immunes were negative.

Sauer and Hambrecht (12) injected *B. pertussis* into the larynx of five healthy young monkeys and observed spontaneous paroxysmal cough after a period of incubation of from one to three weeks.

Rich et al. (13) produced experimental whooping cough in apes. They found that the complement fixation test became positive during the period of cough.

the complement fixation test for gonorrhea. A test with a total volume of 0.5 cc. in each tube was used, the various dilutions of the reagents being added in quantities of 0.1 cc. A 5 per cent suspension of red sheep cells, sensitized with 4 units of hemolytic amboceptor, was employed. In all tests 2 units of complement were used. The sera were diluted 1:5 and inactivated for one-half hour at 56° C. Serum and antigen controls were included in all tests. The reagents were titrated in the customary way. The time of fixation was 40 minutes in the water bath at 37° C. Twenty-five minutes after adding the sensitized cells all test tubes were centrifuged and final readings taken. The antigens were tested against positive antisera prepared by injecting rabbits intravenously with increasing amounts of heat-killed or live pertussis bacilli. Three antigens were used during this study. All sera were examined with Antigen A while Antigen B and C were employed in the latter part of our investigation only. The antigens were prepared from 3 different strains, 1, an old stock strain grown on chocolate agar; 2, an old stock strain grown continuously for several years on Bordet agar by Dr. Povitzki, and 3, a strain recently isolated by Miss Mishulow of this laboratory. It may be stated here that these three strains apparently belong to one type—Type B of the classification of Krumwiede, Mishulow and Oldenbusch (14), since most sera reacting positively with antigens prepared from one of the three strains also gave fixation with antigens derived from the other strains. There were, however, occasional discrepancies, which could be accounted for by quantitative differences in the antigens, due to the method of preparation.

The pertussis bacilli of Strain 1 were grown on three different media.

1. Chocolate agar, prepared by adding about 15 per cent defibrinated horse blood to veal agar, pH 7.4, heating the mixture gradually up to 70° C. and pouring plates at this temperature.

2. Blood veal infusion agar, prepared by adding 30 per cent defibrinated horse blood to veal infusion agar (pH 7.4).

3. Unadjusted Bordet medium, pH 5.8 to 6.1, which was used as 3 per cent agar and to which 30 per cent defibrinated horse blood was added.

No great differences in the antigenicity of Strain 1 were observed in complement fixation tests while growing the organisms on any of the three media, though the authors found that the antigen grown on 30 per cent horse blood veal infusion agar was especially suitable. The two other strains employed in this work were grown on Bordet agar only.

#### *Antigen preparation*

A. Cultures of pertussis bacilli were grown on one of the media described above. After 48 to 72 hours incubation at 37° C., the growth was washed off with a small amount of sterile physiological salt solution (0.85 per cent) and centrifuged for an hour in graduated centrifuge tubes at 3,000 r.p.m. This suspension was mixed

with 50 per cent alcohol in the proportion of 1 cc. of centrifuged organisms to 200 cc. of 50 per cent alcohol. Thus the supernatant fluid was retained in the above mixture. The bacilli were shaken vigorously for about 20 minutes, using glass beads to break them up and then filtered through glass wool. Thus, a homogeneous suspension was obtained. Various amounts of 1 per cent lecithin were added to this stock antigen and tested in preliminary tests. After evaporating the alcohol in an evaporating dish, the bacilli were suspended in an amount of saline solution corresponding to the original volume. For instance, 1 cc. antigen was placed in an evaporating dish and, after evaporating the alcohol, was resuspended in 1 cc. of 0.85 per cent salt solution. The saline was added drop by drop and the rounded end of a test tube was used for bringing the dried material into suspension. In most experiments, an antigen dilution of 1:10 or 1:15 was found suitable for performing the test. In some positive cases the antigens were further diluted 1:20, 30, 40.... In using serum from an immunized rabbit or a strongly positive serum of a patient with whooping cough, one can determine the best dilution of the antigen to be used.

B. This method is similar to that employed by McNeil (18) for the preparation of gonococcus antigen. The 48 hours growth on unadjusted Bordet agar was scraped and put into about 10 times its volume of absolute alcohol. After mixing, it was centrifuged for 10 minutes, the alcohol poured off, fresh alcohol added, put into a water bath and stirred intermittently for 30 minutes at 56° C. The alcohol was poured off, ether added, the mixture stirred intermittently for 30 minutes at room temperature, and then the tubes put into the ice box to settle for 15 minutes. The supernatant fluid was pipetted off, and the tubes centrifuged at the lowest speed for 2 minutes with centrifuge lid open. The rest of the ether was poured off, the tubes returned to the ice box, and covered with a light sterile plug to dry. When thoroughly dried, the material was pulverized and weighed. One gram dried powder was diluted in 200 cc. 0.85 per cent saline. The solution was then heated for 1 hour at 60° C.

The antigen thus prepared was used by the authors in the first series of experiments in the following way: The antigens were diluted 1:15 to 1:20, heated for one-half hour at 56° C. and centrifuged for 10 minutes at low speed to remove coarse particles. In later experiments, however, the antigen was centrifuged at high speed for 40 to 50 minutes and a translucent fluid was obtained which contained the soluble proteins of bacillus pertussis. This fluid was used undiluted in some tests (corresponding to an original dilution 1:200) and in dilution of 1:2 or 1:3 in others. The authors found that treating the fluid with N/20 HCl removed a considerable amount of protein, for after centrifuging, the clear supernatant fluid, on being neutralized with N/20 NaOH, exhibited no antigenic effect.

C. Another method for preparing the antigen was: The 48 to 72 hours' growth of pertussis bacilli on Bordet agar

# COMPLEMENT FIXATION IN PERTUSSIS

was washed off into sterile salt solution (0.85 per cent) using 2 cc. per plate. After the organisms were broken up by vigorous shaking, the suspension was heated for one hour at 60° C., and then centrifuged for 40 to 50 minutes. The slightly opaque supernatant fluid was used as an antigen. While the potency of this antigen was not always as high as that of Antigen A, the specificity was good. This antigen was stored in the ice box and heated for a short time at 56° C. before each test.

## Ice fixation experiments

Gundel (10, 11) repeatedly mentions the fact that fixation at 0° C. as compared with that at 37° C. shows more definitely the specificity of a complement fixation test. Sachs, Klopstock, and Takenomata (15) originally found that unspecific reactions disappear after, 1, heating the serum to 60° to 62° C. or, 2, on performing the tests in the cold (0°). The authors obtained the best results when they incubated the complement fixation tubes at 7° to 8° C. for 3 hours. Experiments with 0° temperatures did not prove as successful as those reported by Gundel. These experiments were made using different antigens with a rabbit immune serum, to test the specificity while varying the incubation period at 0°. There was fixation at 0° C. after 1 hour but the titer was considerably lower than at 37° C. Prolonged incubation for 3 or more hours at 0° C. did not elicit better results inasmuch as the antigen often became anticomplementary. It may be stated that there was a group of several sera, positive at 37° C. and only slightly so at 7° to 8° C. (incubation period 3 hours). Other sera showed almost identical results as compared with the results in the water bath. Whether the reaction in this first group was specific or not could be determined by absorption experiments only.

## Absorption experiments

Good results were obtained using the following method: A heavy suspension of pertussis bacilli was prepared by scraping the growth of 1 or 2 plates into 1 cc. saline. To 0.1 cc. serum, 0.35 cc. saline and 0.05 cc. of the suspension of organisms were added. After mixing the bacilli with the serum dilution, the tubes were incubated at 37° C. for 30 to 40 minutes, centrifuged for one-half hour at high speed, and the supernatant fluid, representing a 1:5 dilution of the serum, was inactivated for 30 minutes at 56° C. and used.

The question now to be considered is the value of the method in the diagnosis of whooping cough. Table I gives a survey of all cases of whooping cough examined. As may be seen, some of the children were examined several times. All of these, except one, showed a definite increase in the titer of complement binding antibodies. Among the 89 cases there were 14 infants. The sera of four of them were positive, one doubtful,

TABLE I  
Survey of 100 tests performed on 89 children suffering from whooping cough

Age years	Number of cases	Result of complement fixation test				
		0	+	++	+++	++++
0 to 1.....	14	9				
1 to 2.....	20	4	1	1	2	2
2 to 3.....	16	3	5	3	5	6
3 to 4.....	9	3	4	3	3	5
4 to 5.....	5	1			2	5
5 to 6.....	8	2			1	3
Older.....	17	2		1	2	3
Totals.....	89	24	10	12	21	33

ful, and the others negative. These findings confirm Gundel's observation that infants rarely form antibodies against pertussis bacilli during the disease. Some of the cases had been coughing for weeks when examined by us, and yet showed a completely negative reaction. On the sera of 75 children 1 year and older, 85 tests were performed which are tabulated in Table II.

TABLE II  
Survey of 85 tests performed on 75 children of more than one year of age suffering from whooping cough

Duration of cough weeks	Result of complement fixation test				
	0	+	++	+++	++++
1					
1½	3	1			
2	1	1	1		
2½	3	1	1		
3	2	1	1		
4	1	1	2	3	4
5	4	1	4	1	
6	1	1	3	3	11
7		1		3	5
8				1	1
13				2	3
Totals	16	7	13	17	32

Regarding the 16 negative results, one must bear in mind the fact that 7 of these tests were performed during the first two weeks of the disease. After the third week of the cough only 6 tests were definitely negative. There were 69 children older than 1 year who were at least in the second week of the cough. From these patients we obtained 53 positive, 5 doubtful, and 11 negative tests.

Considering all the single tests performed on children older than 1 year, the authors found 62 positive, 7 doubtful and 16 negative results. Among the negative and doubtful results there were numerous sera from children coughing but 2 weeks or less. Thus we find 73 per cent positive results among all sera of children older than 1 year, regardless of duration of cough. In all sera examined—including those of infants—the result was positive in 66 per cent only. Among the single specimens examined of children of 1 year of age or older with a cough of more than 2 weeks' duration, about 80 per cent showed a positive complement fixation test. For diagnostic purposes, the test has a limited value because after 2 weeks of cough the clinical and hematological findings are usually sufficient to establish the diagnosis. There were, however, a few cases where the diagnosis could be made very early.

In some children, we tried to compare the serological finding with the clinical severity of the disease, in order to determine whether it has any prognostic significance. Only in a very limited number of cases could definite prognostic conclusions be drawn from the result of the complement fixation test. Severe cases with pneumonia and even fatal cases reacted negatively. Since these patients were infants, this result is not surprising. In other severe cases of pneumonia, the reaction was positive early. A more comprehensive study on a larger number of older infants, who are more likely to form antibodies, might possibly clear up this question.

On the occasion of a small epidemic of whooping cough in a children's home, it was possible to examine the blood of 8 children vaccinated with various whooping cough vaccines a short time previously, and that of 3 control children of the same institution. The 8 children (Cases 1 to 8 of Table III) had been coughing at the time of our examination for about 2½ to 3 weeks only. All of them had been vaccinated about three months prior to the disease.

As may be seen from Table III, the blood of at least 7 of the vaccinated patients showed a considerable titer of complement fixing antibodies in spite of the short persistence of the cough. It is likely that the results have been influenced by the previous vaccination. Since a series of injections given to those children had been finished about 3

TABLE III

*Results of complement fixation test in children with whooping cough who had been previously vaccinated*

Case number	Age	Duration of cough	Result
	years	weeks	
1	4	2½	++++
2	3	3	++++
3	1½	2½	++++
4	2½	2½	++
5	2	2½	++++
6	14 months	2½	++++
7	2	2½	++++
8	2½	2½	++++
Controls			
1	2	3	+++
2	2	3½	+
3	3	3	++++

months before the blood was examined, it is not likely that these children would have shown a positive complement fixation test at the outbreak of the epidemic. As will be demonstrated in another paper, the titers of most patients examined following the vaccination show a definite decline 3 to 4 weeks after the last injection and in several children are completely negative 5 to 6 weeks following the last injection of the whooping cough vaccine. It seems more likely that the antibody response in the group of children mentioned above represents a form of secondary stimulus in a previously vaccinated individual. The 3 control cases do not justify any final statements as to the antibody response in unvaccinated children (with regard to this epidemic), since their number is too small. It is noteworthy, however, that 2 of these 3 children had a definitely lower titer of complement fixing antibodies as compared with the vaccinated patients.

An attempt was made on several occasions to establish the duration of the positive serological findings following the end of the whooping cough period. Since, however, most of the ward patients left the hospital 4 to 8 weeks after the beginning of the disease and failed to reappear in the clinic for bleedings, several months later, the number of cases available to settle this question was insufficient. Therefore, we examined children with various diseases whose history revealed a pertussis a short time previously. Table IV gives a survey of the results of the complement fixation tests on these patients.



TABLE IV

Result of complement fixation tests in children with a positive history of whooping cough

Case number	Age	Time elapsed since whooping cough	Disease	Result
	years			
1	7	3 months		0
2	7	2 months		++++
3	4	2-3 months	Chickenpox	+++
4	5	2-3 months	Chickenpox	+++
5	6	2-3 months		++++
6	7	?	Diphtheria	+++
7	8	1 year		0
8	11½	8-10 months		+++
9	2	1 year		0
10	7	2-3 years		0
11	7	1-2 years	Parotitis	++
12	7	2 years		0
13	5	2-3 years	Scarlet fever	0
14	7	2-3 years	Scarlet fever	++
15	4	2-3 years		0
16	6½	2 years	Measles	0
17	4½	3½ years		++
18	6	5 years		+++

Judging from these observations, the reaction seems to remain positive for about 5 to 8 months after the actual disease. The blood of these children with a history of pertussis several years before ordinarily failed to show fixation. It is not certain whether the positive findings on some of the children (as Case 6 of Table IV, where the exact time interval elapsed since the pertussis is not known, or as Case 17 and 18, where the children had suffered from the disease several years before), were actually caused by the whooping cough. The explanation for the positive findings in these cases may be analogous to that given for the adult cases described below.

Besides the cases represented in Table IV, the authors were able to examine the sera from normal children as well as those from children with various diseases with regard to, 1, previous whooping cough infection, and 2, the specificity of the complement fixation test in children. In cases of measles, the blood was taken during the first 3 to 4 days after the rash appeared, in scarlet fever—usually during the first two weeks of the illness, in tuberculosis—during different stages.

From these experiments, it may be seen that in children free from whooping cough, a positive complement fixation test usually occurs for a limited time only, after the end of the disease. However, weakly positive or doubtful results were observed in some cases where there was no history

TABLE V

Results of complement fixation tests on children with various diseases

Result	Measles	Scarlet fever	Parotitis	Tuberculosis	Varicellae
0.....	11	38	6	38	6
Doubtful	2	2		1	
Positive..	1*	1*	1*		4* (2 cases had pertussis 2 to 3 months before test)

\* Whooping cough in the history.

of pertussis. The various diseases mentioned in Table V, as measles, scarlet fever, chickenpox, tuberculosis, had no influence on the results of the test itself. Nonspecific reactions were of rare occurrence.

Considering the complement fixation tests performed on all children, there was a total of 140 cases, free of pertussis within the last 8 months or not previously immunized. Only 7 of these were positive, 7 doubtful and 126 negative. In other words, 5 per cent were positive and 5 per cent doubtful, irrespective of a history of whooping cough. However, most of these 14 cases had a positive history of pertussis.

It would therefore appear that the complement fixation test in children, if positive, may be used as a diagnostic test. In a number of cases of whooping cough the diagnosis is often difficult, particularly when the patients are seen late in the disease at a time at which the cough plate is likely to be negative and the cough and blood count may not be typical.

An additional advantage of the complement fixation test is the simplicity of the method.

Different results, however, were obtained when we examined the sera of adults. These sera were taken from a number of healthy adults, patients with syphilis, gonorrhea, and various other diseases (as well as sera from volunteers), and also from professional blood donors. The latter group was chosen because it comprises young, healthy men only. Sufficient data as to a history of pertussis during childhood, could not be obtained in all cases.

From Table VI it may be seen that if the ++ results are disregarded, 76 cases out of 562, 13.5 per cent, showed a strongly positive complement fixation.



TABLE VI

*Results of complement fixation tests in healthy adults*

0	+	++	+++	++++	Total
447	8	31	35	41	562

This large percentage is rather surprising and cannot be explained on the basis of an amnesic serological reaction due to pertussis during childhood. As has been stated above, the serological reaction remains positive for a limited time only.

Besides, we tried to find whether there was any relationship between a history of pertussis in these adults and a positive complement fixation test. As far as could be ascertained, no such connection existed. Patients with a history of having had pertussis in childhood would show a completely negative reaction, whereas others who denied having had the disease showed complete fixation.

The next question to be answered was: do the sera of these adults with a positive complement fixation test contain specific amboceptors against Bordet-Gengou bacilli or do they give nonspecific reactions? Absorption tests were performed, according to the method described above, on a number of sera. It was found that the specific antibodies were removed after the sera was absorbed with pertussis bacilli suspensions.

When some of the sera were tested against an influenza bacilli fixing reagent, prepared in the same way as is our Antigen A, the results were negative reactions. At the same time, absorption experiments with pertussis and influenza bacilli demonstrated that sera treated with the latter organisms remained unchanged, while those treated with the pertussis bacilli showed a loss in the antibodies. Table VII gives an example of such an absorption experiment.

TABLE VII

*Result of complement fixation test with Antigen A after absorption of serum with pertussis and influenza bacilli*

Case number	Serum unabsorbed	Absorbed with B. pertussis	Absorbed with B. influenzae
1	++++	0	++++/++++
2	++++	0	++++
3	++/++++	0	++
4	+++	0	++
5	+++	0	+++

In other experiments, a positive complement fixation test carried out at low temperature indicated the specific character of the reaction, according to the conclusions of Gundel (10, 11) and Sachs (15) and coworkers. The method, as performed by us, confirmed the observation that the sera of normal healthy adults contained specific antibodies against pertussis bacilli. We cannot at present explain this finding. Some of these people may have suffered a short time ago from slight attacks of whooping cough, hardly noticeable clinically. Successive examinations of these adult sera for several months could possibly help to decide this question. In one instance, where it was possible to examine the sera of an individual (a laboratory technician) several times during one year, the complement fixation test did not change.

We examined the sera of about 15 adults (nurses, physicians, technicians) who were frequently exposed to whooping cough. The percentage of positive cases was about the same as in our group of normal adults. Further examinations are necessary to decide the question of the increase of complement fixation test by exposure without the development of pertussis disease.

It is a well known fact that adults may acquire whooping cough. We may assume that some of the adults with a positive complement fixation test suffer from subclinical infections which give rise to antibody formation. In children, the infection probably leads to a manifest disease, and positive complement fixation without any connection with whooping cough is rare.

In examining a larger number of adult sera we had the opportunity of studying the sera of 20 maternity patients. In all cases the blood from the umbilical cord was also tested. Sera from three of the women gave positive reactions. In these 3 instances there was a remarkable conformity of the results, in that the specimens of serum from the umbilical cords exhibited reactions almost identical with those obtained with the blood from the corresponding mother.

TABLE VIII

*Results of complement fixation tests of maternal and cord blood*

Case number	Maternal blood	Cord blood
1, W. E.....	++++	+++
2, Ca.....	+++	++
3, Hi.....	++	++
4 to 20.....	Negative	Negative

TABLE IX  
*Antibody response of adults vaccinated with Sauer's vaccine*

	Result of complement fixation test									
Date.....	March 13	March 20		March 27		April 4		April 12		May 8
Antigen dilution.....	1 : 20	1 : 20	1 : 40	1 : 20	1 : 40	1 : 20	1 : 40	1 : 20	1 : 40	1 : 20
Name										
B. Wa.....	0	+++		++++	+++	Same				
B. Br.....	0	++++	+	++++	++	++++	+++	++++	++	
E. Ti.....	0	+++		++++	++++	Same				+++
B. Sm.....	0	0	0		0	0		0		
S. Wt.....	0	++++	+	++++	++	++++	+++			
B. C.....	++			++++	++++	++++	+++	+++		

On March 13 and March 20 all patients received 30 billion organisms.

These results seem to indicate that amboceptors against Bordet-Gengou bacilli can pass through the placenta. This fact, as far as we know, has not been observed as yet. In experiments with actively immunized rabbits, C. Bennholdt-Thomsen (16) has shown that antipertussis antibodies can pass the placenta of these animals.

In a number of adults injected with Sauer's vaccine the serological response was studied. Table IX gives a survey of the results in a few patients so treated.

The antibody response in most vaccinated individuals was immediate. The highest titer of antibodies was found 2 to 3 weeks following the last injection. Since it was not possible to follow up all these cases after vaccination, no final conclusion should be drawn. It is remarkable that from a group of 17 patients treated with Sauer's vaccine, 4 failed to show any antibody response, and this, in spite of the fact that they had received 30 billion organisms or 60 billions, depending on whether they got one or two injections.

No difference was observed in the titers and the rate of antibody formation between those adults having had whooping cough in childhood and those with a negative history.

In another paper to be published soon, more data on whooping cough vaccination will be reported.

#### CONCLUSIONS

1. The complement fixation test in pertussis, as described in this paper, represents a fairly specific serological test giving positive results in about 80 per cent of the cases with whooping cough above 1 year of age and after the second week of cough.

2. The test in most cases becomes positive towards the end of the second week of the disease and usually remains positive for about 5 to 8 months following the cough.

3. In about 90 per cent of normal children the complement fixation test was negative irrespective of a previous history of pertussis. Five per cent gave doubtful and 5 per cent positive results. Thus the complement fixation test, if positive, is of value, as a diagnostic test in whooping cough.

4. In at least 13 per cent of normal healthy adults, positive reactions were also observed. Absorption experiments and fixation tests at low temperature proved these antibodies to be specific. No relation exists between the findings in normal adults and a positive history of pertussis.

5. In comparable tests with maternal blood and that obtained from umbilical cords, the results suggest that specific antibodies against Bordet-Gengou bacilli passed through the placenta.

6. Some data on vaccination of adults with Sauer's vaccine are given in this paper.

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# RESPIRATORY RESPONSE DURING EXERCISE IN PULMONARY FIBROSIS AND EMPHYSEMA

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Numerous tests for cardiac efficiency have been proposed in the past, but relatively little attention has been focused upon breathlessness in patients with respiratory disorders. Fundamental studies of the importance of ventilation in heart disease have been made by Peabody and his fellow workers (1). They demonstrated that the degree of dyspnea was proportional to the reduction in the vital capacity and in the "pulmonary reserve." Harrison and his coworkers (2) recently have studied very thoroughly the pathogenesis of cardiac dyspnea on exertion. They substantiated the work of Peabody in that "a person becomes short of breath when his actual volume of breathing becomes more than a certain fraction of his maximum possible volume and the closer the actual ventilation approaches the maximum possible ventilation the severer dyspnea becomes." In addition, they found that the degree of dyspnea was fairly closely proportional to the expression  $\frac{\text{total ventilation}}{\text{vital capacity}}$  and still more closely related to the ventilation index.

In regard to the pathogenesis of dyspnea in heart disease these investigators believed that it is of nervous origin, due to reflex stimulation of respiration from the muscles, heart and lungs. Christie and Meakins (3) by measuring the pulmonary elasticity and distensibility came to a similar conclusion that breathlessness in patients with heart disease can be explained on the basis of increased sensitivity of the Hering-Breuer reflex due to a decrease in the distensibility of the lungs.

Observations on the respiratory response during exercise on cases other than heart disease have been few in number. Campbell and Poulton (4) investigated this question in cases of chronic bronchitis and found that at rest the minute volume and respiratory rate were increased and during exertion were higher and more prolonged in the group of patients than in the controls.

Attempts have been made to utilize the existing pulmonary reserve by means of the maximum ventilation in order to determine pulmonary function. Sturgis, Peabody, Hall and Fremont-Smith (5) were among the first to obtain the maximum ventilation in response to exercise in normal individuals. Their results showed that the average value for the maximum ventilation was 60.5 liters or approximately 12 times the average breathing volume of such a group at rest. These values were obtained by increasing the respiratory rate to 35 per minute and their tidal volume to 33 per cent of the vital capacity. Hermannsen (6), Jansen, Knipping and Stromberger (7) considered that these values were too low and believed that 100 to 150 liters represented the maximum minute ventilation for male subjects. Marzahn, Gilbeau and Zaeper (8) found in 49 normal individuals that the average maximum ventilation was 70 liters per minute with an average vital capacity of 3.8 liters. In a series of patients with pulmonary emphysema they found the pulmonary reserve greatly reduced.

The respiratory exchange of gases has also been the subject of extensive investigations in heart disease, in an attempt to measure the degree of dyspnea. Herbst (9) studied the amount of oxygen which was absorbed per liter of air inspired, and found that the "utilization coefficient" (amount of oxygen retained by the body out of one liter of inspired air) was reduced in cases of cardiac insufficiency when resting. Knipping, Lewis and Moncrieff (10) and Knipping and Moncrieff (11) applied the "ventilation equivalent for oxygen" (the volume of inspired air that gives up 100 cubic centimeters of oxygen to the body) to cases of cardiac and respiratory diseases. In heart disease, they found that at rest this expression was raised roughly in proportion to the degree of failure present and that on physical exertion there was a further increase, due to the

fact that the minute volume increased out of proportion to the oxygen consumed. In 2 cases of pneumonia the value for the equivalent was elevated, but in isolated cases of pulmonary tuberculosis they concluded that the test was of no particular value.

Moncrieff (12) recently studied 86 patients with chronic respiratory disease. He divided the patients into five groups on the basis of clinical details, and found that as respiratory efficiency was reduced, there was a reduction in the vital capacity and the "dead space" represented a larger portion of the tidal air. The ventilation equivalent for oxygen, however, showed considerable irregularity.

In order to measure the degree of dyspnea by objective means and to understand better the pathogenesis of shortness of breath in patients with chronic pulmonary disease, the present in-

vestigation was undertaken. The ventilation, respiratory rate, tidal volume, oxygen consumption and carbon dioxide production were determined during a standard form of exercise in 20 normal subjects and in 28 patients with cardio-respiratory abnormalities. These results were correlated with anatomical measurements of the lungs and chest and with the gaseous content of the arterial blood. The results are presented in this communication.

#### METHODS

In general, the method in these experiments was to collect the expired air at rest, during five minutes of exercise and 3 minutes thereafter. In order that each subject might perform equal amounts of work, an accurately calibrated stationary bicycle ergometer was used (Figure 1). On the main shaft of the ergometer were mounted a heavy fly wheel and an electrical brake. An automobile speedometer was used as a speed indicator,

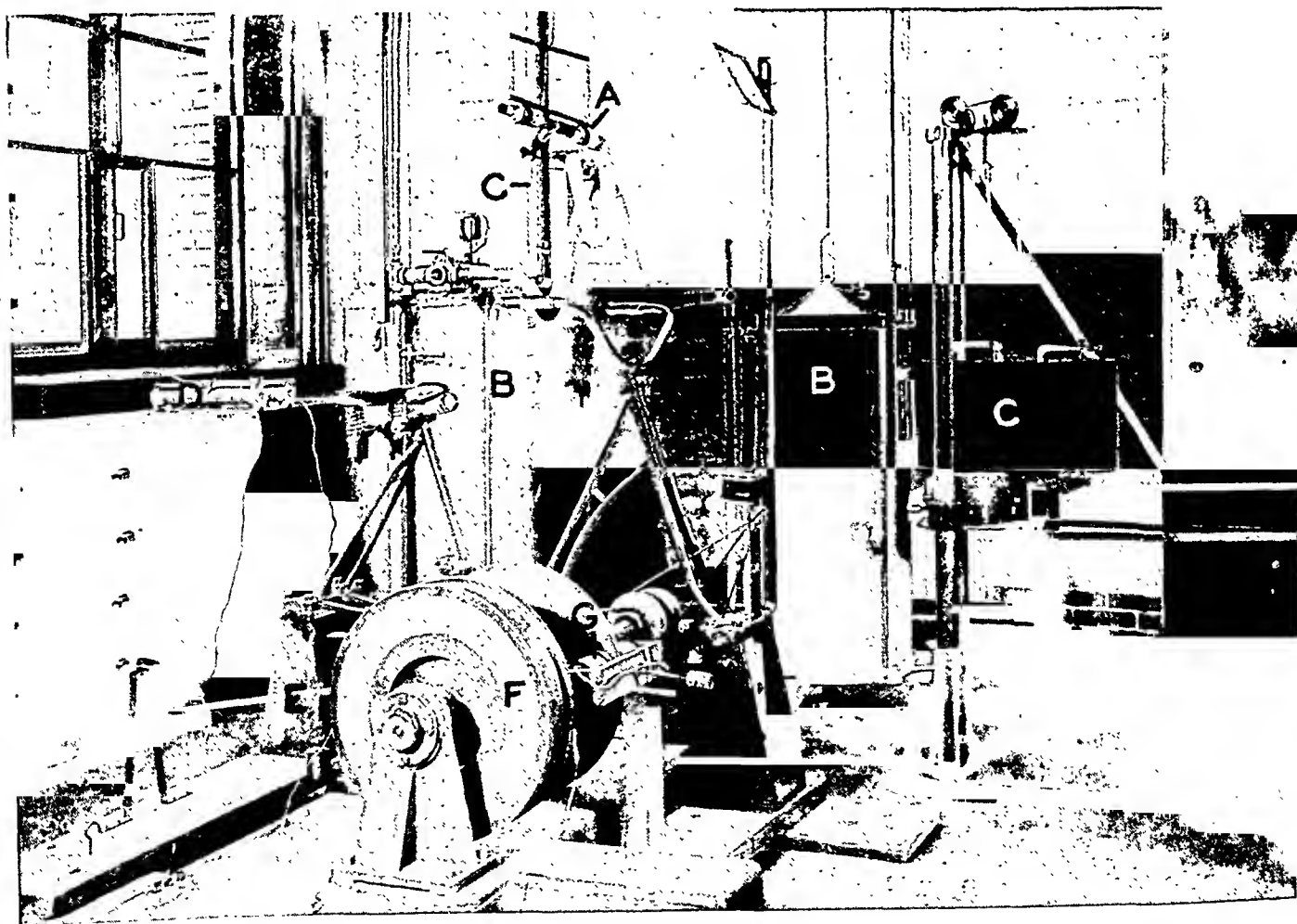


FIG. 1. BICYCLE ERGOMETER.

A. Mouthpiece with a set of flutter valves. B. Tissot spirometers. C. Recording drums. D. Rheostats to control the current through the electromagnets of the electrical brake. E. Lever arm of the electrical brake. F. Fly wheel. G. Electrical brake.

the face of which could be readily seen by the subject by means of a mirror. Above the bicycle and suspended from the ceiling of the room was a mouthpiece with a set of flutter valves, so arranged that, during inspiration, air was withdrawn from the room and during expiration it was directed by means of an expiratory valve through a large corrugated rubber tubing to a three-way valve. The connections from the valve were arranged so that the expired air could be collected either in a 150 liter or in a 550 liter Tissot spirometer. Both spirometers were equipped with recording pens and revolving drums.

The course of the experiments was as follows: the subject rested on a chair close to the ergometer for 20 minutes, following which he rested an additional 5 minutes on the seat of the bicycle. The mouthpiece was then connected and the nose clip was adjusted. The three-way valve was turned so that the air was collected for 4 or 5 minutes in the small spirometer. After this period the air was expelled and the procedure repeated. Finally, during the third collection, the period was accurately clocked by a stop watch and the number and volume of each respiration were recorded on the revolving drum. At the end of this period the mouthpiece was disconnected. Samples of the expired air were then withdrawn from the spirometer and analyzed later for carbon dioxide and oxygen according to the method described by Van Slyke and Sendroy (13). Duplicate determinations were made on each sample.

The mouthpiece was then reconnected and during rest the expired air was collected in the large spirometer for one minute. Exactly at the end of that time the subject was given the command "ride." He was instructed beforehand to pedal so that the speedometer registered 30 miles per hour, which corresponded to 67 revolutions of the pedals and 167.5 revolutions of the shaft per minute. At this speed the resistance of the ergometer without a load was 108 kilogrammeters per minute. Weights were so adjusted on the lever arm of the electrical brake that the sum of the resistance of the ergometer and the effect of the brake equalled 300 kilogrammeters per minute. The subject continued to pedal for five minutes, at the end of which time he was given the command "stop." The air was collected in the spirometers during the five minutes of exercise and three minutes of rest thereafter.

After the subject rested for 30 minutes, greater amounts of work were performed for a similar period of time. In the final run, the subject was asked to ride the bicycle as long and as fast as possible, until a state of complete exhaustion was reached.

At the completion of each exercise the subject was questioned concerning respiratory distress, muscle fatigue and other symptoms.

The records were measured and the ventilation and respiratory rate and average tidal volume were determined for each one-half minute. The volumes were reduced to standard temperature and barometric pressure.

The maximum minute ventilation was obtained by averaging the ventilation during the last one and one-half minutes of maximum exertion, and the pulmonary

reserve determined by subtracting the average minute ventilation at rest from the maximum minute ventilation.

The methods of determining the residual air and other components of the total pulmonary capacity as well as the methods of radiographic technique and measurements have been described elsewhere (14, 15). The vital capacity was determined both in the lying and sitting positions.

Blood was obtained from the radial artery and stored over mercury in ice water until the analyses were made. Both before and immediately after exercise the blood was secured while the patient was in a semi-recumbent position.

#### MATERIAL

This series includes 20 normal subjects, between the ages of 18 and 48 years. The history and physical and fluoroscopic examinations revealed that they were free of any discernible disease. Of the remaining 28 cases, 11 had varying degrees of pulmonary fibrosis, most of which were due to the inhalation of silica; 8 cases of emphysema, all of which except one had histories of repeated attacks of bronchial asthma and 4 of these had co-existing pulmonary fibrosis; 2 cases of chronic bronchitis; 2 cases with histories of attacks of bronchial asthma since childhood, but at the time of examination apparently normal except for clubbing of the fingers in one (H. S.); and five cases of rheumatic heart disease (Table I).

Complete medical histories were taken and physical examinations were made in each case. None of the patients with pulmonary fibrosis had symptoms or signs of active pulmonary tuberculosis, but many had superimposed bronchial infections and one (S. B.) had repeated attacks of bronchopneumonia during the past 3 years. Two of the patients (S. W., J. B.) had definite evidence of complicating heart disease. Cough and dyspnea on exertion were the two most common symptoms, while clubbing of the fingers was a rarity. The cases of fibrosis were grouped according to the lesion as seen by the radiograph and recently described by McCann, Hurtado, Kaltreider and Fray (16). Varying degrees of emphysema, as evidenced by the increase in the residual air, was shown by the patients with pulmonary emphysema. All the patients with heart disease had mitral stenosis and insufficiency of rheumatic origin and one (S. V.) had in addition aortic stenosis and incompetency. Two of the cases (W. C., J. W.) had mild congestive failure, while

TABLE I

*The pulmonary capacity, expansion of the chest and arterial blood in pulmonary fibrosis and emphysema and heart disease*

Age	Surface area	Total capacity	Vital capacity		Residual air	Per cent of predicted			Area maximum expiration		Electrocardiogram	Diagnosis
			Ly-ing	Sit-ting		Total capacity	Vital capacity	Re-sidual air	per cent	per cent		
years	square meters	liters	liters	liters	per cent	per cent	per cent	per cent	per cent	per cent		
18-35	6.13	4.78	4.99	1.36	22.0	80-120	80-120	60-140	61	62		Normal
35-63	5.57	4.07	4.30	1.53	27.0							
Fibrosis												
1. R.D....	1.75	6.39	4.10	4.30	2.29	114	100	150	62		Normal	Pulmonary fibrosis, Group I†
2. L.S....	1.82	5.01	3.24	3.60	1.77	98	87	127	61		L.A.D.*	Pulmonary fibrosis, Group I
3. W.S....	1.70	6.14	3.40	3.90	2.74	105	80	173	78		Normal	Pulmonary fibrosis, Group III
4. R.A....	1.81	4.17	2.70	3.00	1.47	77	68	99	74		Ventricular extrasystoles	Pulmonary fibrosis, Group III
5. S.B....	1.35	3.44	1.74	2.96	1.70	65	45	117	75		L.A.D.	Pulmonary fibrosis, Group III
6. R.P....	1.71	4.23	2.86	3.20	1.37	72	63	93	71		Normal	Pulmonary fibrosis, Group III
7. D.V....	1.81	4.05	2.84	3.30	1.21	72	65	98	70		L.A.D.	Pulmonary fibrosis, Group IV
8. D.D....	1.63	3.84	2.10	2.45	1.74	65	49	109	73			Pulmonary fibrosis, Group IV
9. M.M....	1.70	3.95	2.34	2.39	1.61	79	64	118	78		Normal	Pulmonary fibrosis, Group IV
10. S.W....	1.74	3.98	1.55	1.80	2.43	73	39	165	75		R.A.D.*	Pulmonary fibrosis, Group VI, polycythemia
11. G.H....	1.83	2.52	1.00	1.00	1.52	51	28	113	92		Normal	Pulmonary fibrosis, Group IV
Average...		4.34	2.53	2.90	1.80	80	63	124	73			
Emphysema												
12. J.B....	1.76	6.09	1.96	1.90	4.13	94	41	233	82		Normal	Pulmonary fibrosis and emphysema, history of asthma
13. E.W....	1.98	6.13	2.82	2.70	3.31	78	49	155	83		L.A.D.	Pulmonary fibrosis and emphysema, history of asthma
14. M.M....	1.84	6.10	3.85	3.90	2.25	103	90	141	65		Normal	Pulmonary emphysema
15. J.H....	1.63	5.59	2.74	3.10	2.83	95	64	177	74		Normal	Pulmonary fibrosis and emphysema, history of asthma
16. S.R....	1.80	5.90	3.30	3.00	2.60	99	76	160	72		M.D.*	Pulmonary emphysema, history of asthma
17. F.S....	1.94	8.06	3.64	3.40	4.42	120	75	243	70		Normal	Pulmonary fibrosis and emphysema, history of asthma
18. H.H....	1.93	7.56	3.40	3.30	4.16	118	70	229	78		Normal	Pulmonary fibrosis and emphysema, history of asthma
19. A.D....	1.75	6.27	2.12	2.20	4.15	94	44	232	87			
Average...		6.46	2.98	2.94	3.48	100	64	196	70			
Heart disease												
20. R.C....	1.70	5.30	3.20	3.35		82	82	122	83		Chronic bronchitis	Chronic bronchitis
21. E.N....	1.57	5.30	3.70	3.90	1.90	110	106	123	74		History of asthma, no emphysema	History of asthma, no emphysema
22. J.R....	1.34	6.08	4.00	4.40	1.78	100	93	136	69			
23. H.S....	1.79		4.30	4.45					65			
Heart disease												
24. J.O....	1.66	5.06	4.00	4.20	1.06	95	97	91	61		Normal	Rheumatic heart disease, mitral stenosis and insufficiency
25. S.V....	1.56	3.94	2.90	3.30	1.04	78	74	94	67		Normal	Rheumatic heart disease, mitral stenosis and insufficiency, aortic stenosis and insufficiency
26. J.W....	1.59	5.40	3.35	3.35	2.05	99	84	138	84		R.A.D., 1st degree A-V block, M.D.	Rheumatic heart disease, mitral stenosis and insufficiency
27. W.C....	1.76	4.86	2.95	3.85	1.91	82	69	119	74		M.D., 1st degree A-V block	Rheumatic heart disease, mitral stenosis and insufficiency
28. A.B....	1.92	4.49	2.97	3.15	1.52	71	60	109	70		R.A.D., M.D.	Rheumatic heart disease, mitral stenosis and insufficiency
Average...		4.75	3.23	3.57	1.52	85	77	110	72			

\* L.A.D. = Left axis deviation. R.A.D. = Right axis deviation. M.D. = Myocardial damage.

† Grouped according to the nature and the extent of radiographic lesions. I. Increased linear markings in the lung fields. III. Simple nodular fibrosis. IV. Agglomeration of nodules. VI. Fine, diffuse reticular fibrosis without nodulation.

one (A. B.) had moderate failure. The other two cases were well compensated.

The individuals in this series were divided into 4 groups (A, B, C, D), according to disability assessed from their histories. The individuals investigated have been known to us for a period of from one to four years, and we feel that they have been classified as accurately as is possible from clinical details alone.

## RESULTS

### *Pulmonary capacity and expansion of the chest.*

Details of the various subdivisions of the pulmonary capacity are presented in Table I. The values given here substantiate those previously reported from this clinic (17, 18). Briefly, in cases of pulmonary fibrosis there was a moderate decrease in the total capacity, marked decrease in the vital capacity and a slight rise in the residual

air, resulting in a slight increase in the ratio  $\frac{\text{residual air}}{\text{total capacity}} \times 100$ . As the degree of fibrosis increased, these alterations were accentuated. The patients with pulmonary emphysema showed a normal value for the total capacity, while the vital capacity was greatly reduced and the residual air correspondingly increased. The values for the pulmonary capacity in patients with heart disease were very similar to those with pulmonary fibrosis, i.e., a slight decrease in the total capacity, moderate decrease in the vital capacity while the residual air was slightly increased.

The ability to expand the chest in the three major groups was greatly diminished as evidenced by the increase in the ratio

$$\frac{\text{area at maximum expiration}}{\text{area at maximum inspiration}} \times 100.$$

### *Pulmonary ventilation, respiratory rate and*

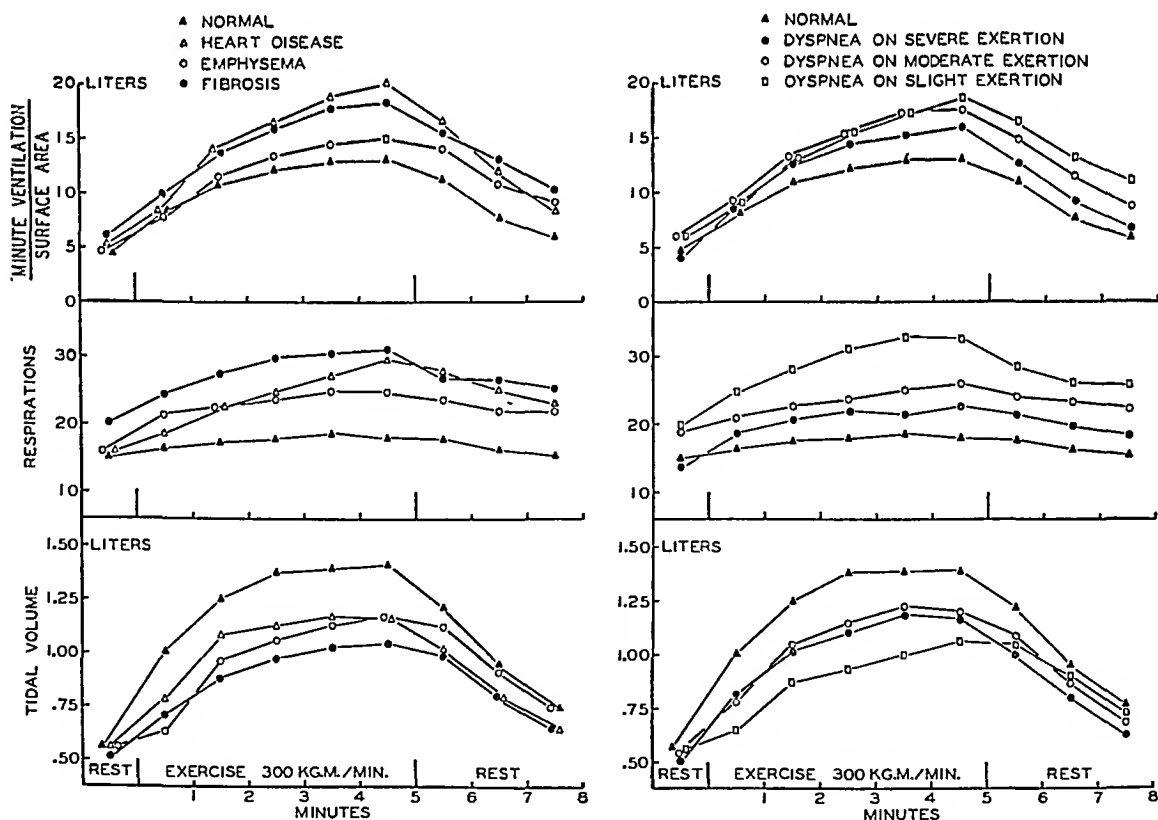


FIG. 2. THE MINUTE VENTILATION, RESPIRATORY RATE AND TIDAL VOLUME AT REST AND DURING EXERCISE OF NORMAL SUBJECTS AND OF PATIENTS WITH PULMONARY FIBROSIS, OBSTRUCTIVE EMPHYSEMA AND HEART DISEASE.



*tidal volume.* The patients with cardiorespiratory disorders are contrasted with the normal subjects in respect to minute volume per square meter of body surface area, respiratory rate and tidal volume (Figure 2). At rest, the average minute volume of the patients with fibrosis was 6.1 liters or 36 per cent higher than the average value for the normals. The average minute ventilation for the patients with heart disease was 16 per cent higher than the normal controls, while that of individuals with emphysema was 3 per cent higher.

As a result of moderate exercise (300 kilogrammeters per minute for five minutes), the minute volume was increased in all groups, but it reached a considerably higher level in the patients with heart disease and those with fibrosis than it did in the patients with emphysema and the controls (Figure 2). During the fifth and last minute of exercise the average minute volumes for the cases with fibrosis and heart disease were 41 and 54 per cent respectively higher than the controls, while in the patients with emphysema it was only 16 per cent higher.

After three minutes of rest the average values for the minute ventilation were much higher in the three groups than in the normal subjects.

The respiratory rate at rest was considerably higher in the patients with fibrosis than the normals, while the rates of the other two groups were only slightly higher. During exercise, the rate increased in all groups, but the increment was much greater in the patients with heart disease and fibrosis than in the other two groups. At the end of three minutes of rest, the respiratory rate remained elevated in the cases of cardiorespiratory disease, while it returned to the resting level in the control group.

There were equally marked changes in the tidal volume during exertion in the four groups. At rest, the volume of each respiration was essentially the same for the normal subjects and for those with heart disease and emphysema, while the individuals with fibrosis had somewhat smaller tidal volume. During exercise the increase in the tidal volume was greater in the controls than in the patients with cardiac or pulmonary disorders. The smallest increase was in the patients with fibrosis.

An analysis of the second part of Figure 2 shows that in patients who develop dyspnea on moderate or slight exertion, the minute volume

and the respiratory rate were greater while the tidal volume was smaller during exercise than in those patients and normal subjects who develop shortness of breath only on severe exertion.

It appears, then, from these results that there is a tendency for higher minute volumes to be associated with higher respiratory rates and smaller tidal volumes. These observations corroborate the studies of Peabody and Sturgis (19) on heart disease. The close similarity of the patients with pulmonary fibrosis and those with heart disease in respect to ventilation during exercise is striking.

*The relation of the expression,  $\frac{\text{total ventilation}}{\text{vital capacity}}$  to dyspnea.* In Table II are presented the results of the total ventilation for 5 minutes of exercise and the following 3 minutes of rest per square meter of body surface area. While performing 1500 kilogrammeters in 5 minutes, the average value for the 20 normal subjects was 83 liters with extremes of 60 and 104 liters per square meter. In 11 cases of fibrosis the total ventilation was greater than that of the controls with an average value of 114 and extremes of 88 and 173 liters per square meter. Similar values were obtained for the 5 patients with heart disease, while 8 patients with emphysema showed values midway between the normal subjects and those with heart disease and fibrosis.

When the patients are arranged according to their disability as estimated from the history, the values for  $\frac{\text{total ventilation}}{\text{surface area}}$  increased as dyspnea is more readily produced. The average value for the subjects in Group B, none of whom complained of dyspnea while performing 1500 kilogrammeters in 5 minutes, was 96, with extreme variations of 74 and 118 liters. In Group C all the individuals except two (R. P. and D. V.) complained of breathlessness while performing this amount of exercise, and the average value for the group was 108 liters. All the patients in Group D noticed dyspnea, and the average value was 114 liters. There was considerable overlapping of the values for the total ventilation in this series as shown in Table III. Thus it appears that  $\frac{\text{total ventilation}}{\text{surface area}}$  is only roughly proportional to the degree of dyspnea. These results substan-

TABLE II  
The response of ventilation during moderate and severe exertion

	Minute ventilation Vital capacity (rest)	300 kilograms per minute for 5 minutes										Maximum minute ventilation		Pulmonary reserve	
		Dysp- nea	Total ventilation Surface area	Total ventilation Vital capacity	Ventila- tion index	Minute ventilation 4th and 5th minute (average)		Minute ventilation 4th and 5th minute (average) M.M.V.† (predicted) × 100	Observed liters 49-107	Pre- dicted liters 71 54-88	Observed liters 63 32-98	Pre- dicted liters 63 47-81			
						per cent									
Normal	1.70 1.2-2.6		83 60-104	32 21-48	33 23-45	35 27-46	34 21-53								
Fibrosis															
1. R. D. ....	1.9	0	109	45	47	51	48		60	63	53	55			
2. L. S. ....	2.2	0	82	42	38		49			53		45			
3. W. S. ....	1.6	0	118	51	59		61			57		51			
4. R. A. ....	4.0	++	94	57	59	70	59		37	44	25	32			
5. S. B. ....	4.4	++	173	79	85	98	80		36	44	23	30			
6. R. P. ....	2.9	0	101	54	54	49	55		53	47	44	38			
7. D. V. ....	3.6	++	107	59	56	48	64		65	49	53	37			
8. D. E. ....		++	112	73	82	73	81		40	36		25			
9. M. M. ....	4.2	++	157	111	117	75	121		56	35	46	13			
10. S. W. ....	7.7	++	88*	85*	75*	100*	90*		24	15	11	2			
11. G. H. ....	12.6	++													
Average.	4.5		114	66	67	71	71		46	43	36	33			
Emphysema															
12. J. B. ....	2.2	++	65*	60*	57*		60*		35	28	29	34			
13. E. W. ....	1.7	++	83	61	65	88	79			40		51			
14. M. M. ....	4.0	0	140	73	73	90	41		39	46	27	33			
15. J. H. ....	3.3	++	91	55	54	74	55		31	44	25	44			
16. S. R. ....	1.8	++	81	46	42	65	46		40	50	32	40			
17. F. S. ....	2.5	++	86	50	49	93	55		38	32	28	22			
18. H. H. ....	4.5	++	132	105	113		108								
19. A. D. ....															
Average.	2.9		95	61	61	82	65		37	43	28	37			
20. R. C. ....	2.0	0	86	44	45	43	39		56	49	50	43			
21. E. N. ....	1.8	0	106	43	47	52	49		53	57	47	50			
22. J. R. ....	2.1	0	102	31	34	41	36		53	65	46	58			
23. H. S. ....	1.6	0	74	30	30		33								
Heart disease															
24. J. O. ....	1.6	0	94	37	37		41			62		55			
25. S. V. ....	2.0	0	102	48	49		53			49		42			
26. J. W. ....	2.0	++	113	54	56		70			49		42			
27. W. C. ....	3.5	++	117	53	57		61			57		43			
28. A. B. ....	3.6	++	148	90	94		100			46		35			
Average.	2.5		115	56	59		65			53		43			

\* Less than 300 kilograms per minute.  
† M.M.V. = maximum minute ventilation.

TABLE III  
Ventilatory response during exercise related to the degree of dyspnea

Group†	Vital capacity, per cent of predicted	Residual air Total capacity ×100	Area maximum expiration Area maximum inspiration ×100	Oxygen saturation of arterial blood	300 kilograms per minute for 5 minutes						Pulmonary reserve		Ventilation equivalent	
					Dyspnea	Total ventilation Surface area	Total ventilation Vital capacity	Minute ventilation 4th and 5th minute (average) M.M.V.† (observed) ×100	Minute ventilation 4th and 5th minute (average) M.M.V.† (predicted) ×100	Observed liters	Pre-dicted liters	Rest	300 kilo-gram-meters	
A	per cent 100 80-120	per cent 25 15-40	per cent 62 54-73	per cent	0	liters 83 60-104	32 21-48	per cent 35 27-46	per cent 34 21-53	63 32-98	63 47-81	2.40 1.83-3.98	1.86 1.37-2.20	
B					0									
1. R. D.	100	36	62	94.9	0	109	45	51	48	53	55	3.39	2.58	
2. W. S.	80	45	78	91.0	0	118	51		61		51	2.59	2.79	
3. L. S.	87	35	61	93.9	0	82	42		49		45	2.54	2.14	
14. M. M.	90	37	65	96.4	0	83	39		41		51	2.30	2.05	
24. J. O.	97	21	61	94.5	0	94	37		41		55	2.52	2.26	
25. S. V.	74	26	67	91.9	0	102	48		53		42	2.15	2.17	
20. R. C.	82		83		0	86	44	43	39	50	43	2.11	1.85	
21. E. N.	106	30	74		0	106	43	52	49	47	50	2.81	2.51	
22. J. R.	123		69		0	102	31		36	46	56	3.55	1.96	
23. H. S.	93	29	65	93.6	0	74	30	41	33	46	58	2.76	1.72	
Average.	93	32	69	93.7		96	41	47	45	49	51	2.67	2.20	
C					+									
26. J. W.	84	38	84	94.0	+	113	54		70	44	42	2.87	2.92	
6. R. P.	73	32	71	95.0	0	101	54	49	55	53	38	2.76	2.00	
7. D. V.	65	30	70	94.5	+	107	59	48	61	25	37	2.23	2.37	
4. R. A.	68	35	74	89.6	++	94	57	70	59	27	32	3.52	2.31	
15. J. H.	64	51	74	86.9	++	140	73	90	78	27	33	3.37	3.68	
17. F. S.	75	55	70	90.8	++	81	46	74	46	25	44	2.73	2.22	
27. W. C.	69	39	74		+	117	53		61	25	43	4.08	3.59	
Average.	71	40	74	91.8		108	57	66	62	35	38	3.12	2.73	
D					++									
5. S. B.	45	49	75	85.8	++	173	79	98	80	23	30	4.40	2.73	
18. D. E.	49	45	73	84.7	++	112	73	73	81	46	25	3.55	2.93	
9. M. M.	64	41	78	93.0	++	157	111	75	121	46	13	3.07	2.16	
10. S. W.	39	61	75	79.9	++	88*	85*	100	90	11	2			
11. G. H.	28	60	92	81.0	++	83	61	88	79	29	34	2.97	2.37	
13. E. W.	49	54	83	91.0	++	65*	60*		60					
12. J. B.	41	68	82	83.9	++	91	55		55					
16. S. R.	76	44	72	95.2	++	91	105	93	108	28	34	3.59	2.31	
10. A. D.	44	66	87	80.0	++	132	90	65	55	32	22	3.58	2.97	
18. H. H.	70	55	78	92.0	++	86	50		55	32	40	2.33	2.21	
28. A. B.	60	34	76	89.7	++	148	90		100	35	35	3.37	5.74	
Average.	51	52	79	86.9		114	77	85	83	28	26	3.36	3.06	

\* Less than 300 kilograms per minute.

† B. Dyspnea on severe exertion. C. Dyspnea on moderate exertion. D. Dyspnea on slight exertion.

‡ M.M.V. = maximum minute ventilation.

tiate the findings of Harrison and his coworkers (2) in heart disease.

Peabody (1) called attention to the relationship between the vital capacity and the degree of dyspnea in heart disease. It has been shown by Hurtado et al. (17, 18) that this relationship holds true in cases of pulmonary emphysema and fibrosis. In this series of cases the relationship between the vital capacity and the ease with which dyspnea is produced is shown in Table III. When the observed vital capacity was less than 70 per cent of the predicted, by the formula of Hurtado and Fray (15), dyspnea was nearly always produced by moderate physical exertion.

Harrison and others (20) found that the degree of dyspnea in heart disease was more closely related to the expression  $\frac{\text{total ventilation}}{\text{vital capacity}}$  than to

either of these factors alone. The results of this series of cases are very similar to those reported by these investigators. The values for this expression while performing work are given in Table II and Figure 3. At any value below 40 for the expression  $\frac{\text{total ventilation}}{\text{vital capacity}}$  none of the individuals complained of dyspnea, while at values between 40 and 66 mild dyspnea was noted. The average value at which breathlessness was noted in all the normal subjects was 51. As the values for this expression increased the degree of dyspnea became more marked, so that when they approximated 80 moderate to severe distress was experienced. None of the normal subjects noticed shortness of breath while performing 300 kilogrammeters per minute for 5 minutes and all the values were below 48. As the amount of

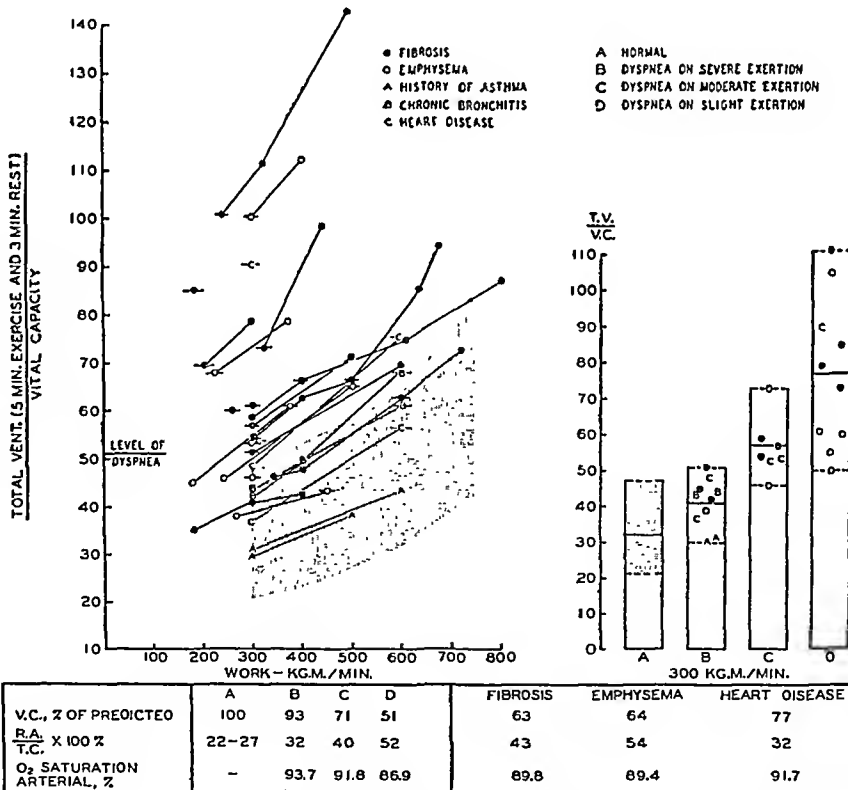


FIG. 3. RELATIONSHIPS BETWEEN THE EXPRESSION,  $\frac{\text{TOTAL VENTILATION}}{\text{VITAL CAPACITY}}$ , WORK AND THE LEVEL OF DYSPNEA.

The area shaded with dots represents the range of values for the normal subjects. The horizontal lines through the various symbols designate the value at which dyspnea was noticed.

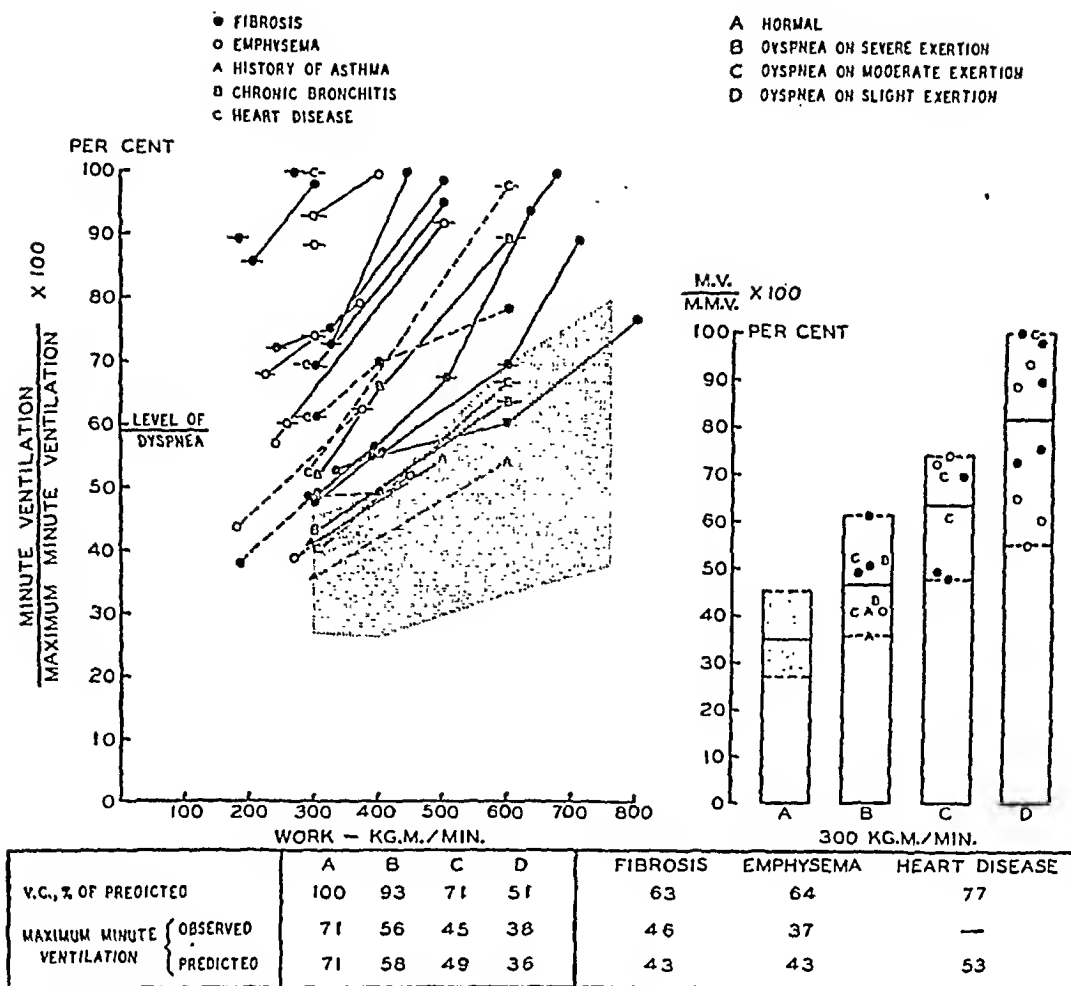


FIG. 4. RELATIONSHIPS BETWEEN THE RATIO  $\frac{\text{MINUTE VENTILATION}}{\text{MAXIMUM MINUTE VENTILATION}} \times 100$ , WORK AND THE LEVEL OF DYSPNEA.

The dotted area represents the range of values for the normal subjects. The horizontal lines at the various symbols designate the value at which dyspnea was noticed. The observed values for the maximum minute ventilation were used in the ratio, where the symbols are connected by unbroken lines, while the predicted values were used where the symbols are joined by broken lines.

tained in patients with heart disease. However, if this volume is predicted from the observed vital capacity and the minute ventilation is then expressed as percentage of the maximal ventilation, the values increased during moderate exertion from 41 per cent in the case of J. O., who was compensated, to 100 per cent in the case of A. B. who was in a state of congestive failure. The values for the pulmonary reserve ranged from 62 liters in the former to 46 in the latter.

It is of interest to compare the pulmonary reserve of these individuals when they are grouped according to the disability as estimated from their clinical findings. This comparison is shown in Figure 5. The maximum minute ventilation of Group A (normal subjects) was 71 liters and the

minute volume at rest was 8 liters or a reserve of 63 liters. As the clinical condition progressively became worse, the pulmonary reserve decreased, so that the individuals in Group D (dyspnea on slight exertion) had a maximum minute ventilation of only 38 liters and a reserve of 28 liters. When the minute volume at rest was expressed as percentage of the maximum volume, there was a gradual increase in the percentage from 12.4 in the normal subjects to 25.7 in the individuals who developed dyspnea on slight exertion. It is evident that the reduction in pulmonary reserve in those more severely disabled, was due to two factors, reduction in the maximum minute ventilation and an increase in the ventilation at rest.

During moderate physical exertion the normal subjects utilized only 35 per cent of the maximal ventilation. None of them complained of dyspnea as they still retained 24 per cent of their symptomatic reserve. Although the individuals in Group B were actually using 12 per cent more of their maximal ventilation than the controls, dyspnea was not noticed as they still had an symptomatic reserve of 12 per cent. The situation, however, was quite different in Groups C and D. Here the individuals during moderate exertion had not only exhausted their reserve without symptoms but had greatly encroached upon their reserve with symptoms, and shortness of breath was complained of by all but two patients in Group C and by all in Group D.

Similar results were found when the values for the maximum minute ventilation were predicted in each case, Figure 5.

*The ventilation equivalent for oxygen.* Forty-six subjects were studied in reference to the ventilation per 100 cc., oxygen absorbed under conditions of rest, and during the fifth minute of moderate physical exertion (Tables III and IV). The average value for the ventilation of normal individuals was 2.40 liters per 100 cc. oxygen absorbed, with extremes of 1.83 and 3.98 liters. These values are in close agreement with those reported by Knipping and Moncrieff (11). The average values for this equivalent were increased in all groups of patients with cardiorespiratory disease. The average value in the group with fibrosis was 3.15 with extremes of 2.23 and 4.40 liters, while in that with emphysema it was slightly lower, 2.98 liters. Equally high values were found in the group of five patients with heart disease.

During moderate physical activity the ventila-

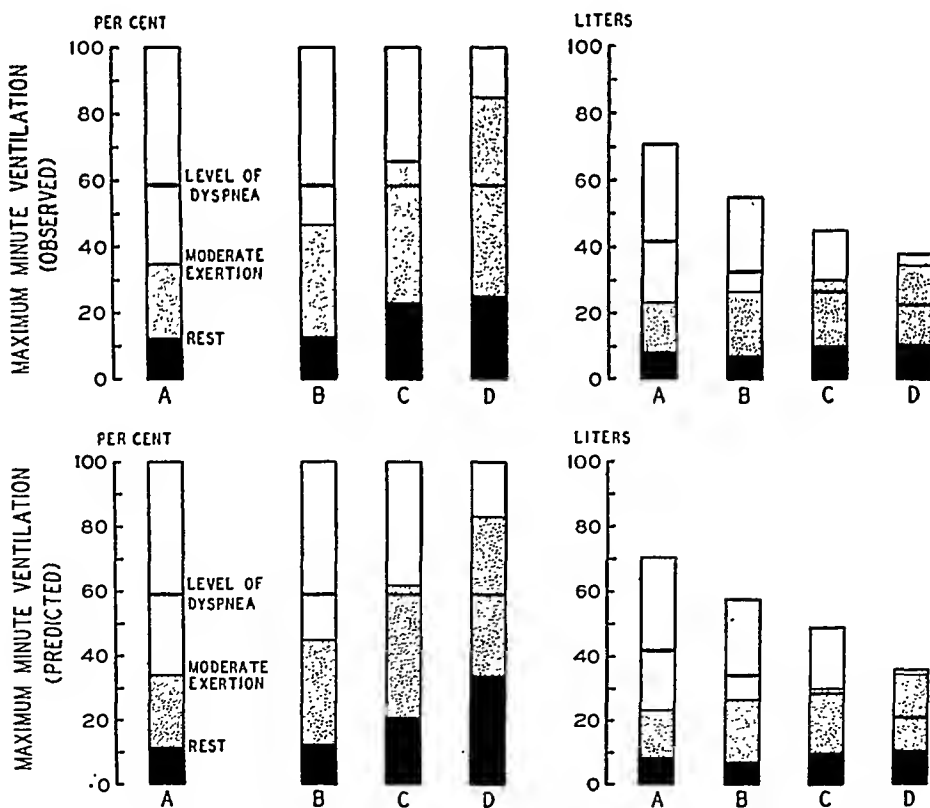


FIG. 5. THE PULMONARY RESERVE EXPRESSED IN RELATIVE AND ABSOLUTE VALUES FOR NORMAL SUBJECTS AND CASES OF CHRONIC PULMONARY DISEASE AND HEART DISEASE.

Moderate exertion refers to 300 kilogrammeters per minute for five minutes. *A* designates normal subjects; *B*, patients who develop dyspnea on severe exertion; *C*, dyspnea on moderate exertion; and *D*, dyspnea on slight exertion.

TABLE IV  
Ventilation equivalent for oxygen

	Ventilation equivalent*	
	Rest	Exercise
Normal	2.40 1.83-3.98	1.86 1.37-2.20
Fibrosis		
1. R. D.....	3.39	2.58
2. L. S.....	2.54	2.14
3. W. S.....	2.59	2.79
4. R. A.....	3.82	2.31
5. S. B.....	4.40	2.73
6. R. P.....	2.76	2.00
7. D. V.....	2.23	2.37
8. D. E.....		2.93
9. M. M.....	3.55	4.16
10. S. W.....	3.07	2.16
11. G. H.....		
Average.....	3.15	2.62
Emphysema		
12. J. B.....		2.37
13. E. W.....	2.97	2.05
14. M. M.....	2.30	3.68
15. J. H.....	3.37	2.31
16. S. R.....	3.59	2.22
17. F. S.....	2.73	2.21
18. H. H.....	2.33	2.97
19. A. D.....	3.58	
Average.....	2.98	2.54
20. R. C.....	2.11	1.85
21. E. N.....	2.81	2.51
22. J. R.....	3.55	1.96
23. H. S.....	2.76	1.72
Heart disease		
24. J. O.....	2.52	2.26
25. S. V.....	2.15	2.17
26. J. W.....	2.87	2.92
27. W. C.....	4.08	3.59
28. A. B.....	3.37	5.74
Average.....	3.00	3.34

\* Ventilation in liters per 100 cc. O<sub>2</sub> absorbed.

tion equivalent for oxygen fell in all groups except one, patients with heart disease, in which the values increased from 3.0 to 3.34 liters. Knipping and Moncrieff found that during physical exertion the values for the equivalent remained the same in normal subjects, but increased in patients with heart disease. In the patient A. B., who had decompensated rheumatic heart disease, the value rose from 3.37 at rest to 5.74 liters during exercise, while in the patient J. O., who developed dyspnea only on severe exertion, the value fell from 2.52 at rest to 2.26 liters during exertion.

An analysis of the results in these patients with pulmonary disease when they are arranged in

groups according to their disability, showed that at rest the average value for this equivalent increased from 2.4 liters in Group A, to 3.36 liters in Group D. Similar changes were noted during moderate exertion. Although the average values increased as the clinical condition became worse, there were marked individual variations and the overlapping among the several groups was marked. It appears that it is only a fair measure of the degree of dyspnea.

*Oxygen in the arterial blood before and after exercise.* Blood was obtained from the radial artery at rest and immediately after moderate severe exercise. The results in 9 cases (7 cases with pulmonary fibrosis and 2 with emphysema and fibrosis) are presented in Table V. In all

TABLE V  
Arterial blood before and immediately after exercise

	Carbon dioxide content		Oxygen content		Oxygen capacity		Oxygen saturation		Hemoglobin per 100 cc.	
	Be-fore	After	Be-fore	After	Be-fore	After	Be-fore	After	Be-fore	After
	vol-umes per cent	vol-umes per cent	vol-umes per cent	vol-umes per cent	vol-umes per cent	vol-umes per cent	per cent	per cent	grams	grams
5. S. B....	44.3	39.4	16.6	17.9	17.0	18.7	92.9	95.7	13.3	14.0
6. R. P....	47.6	34.8	17.5	18.7	18.6	20.3	94.0	92.1	13.9	15.2
8. D. E....	49.5	39.9	15.1	17.9	17.8	19.2	84.7	93.2	13.3	14.3
9. M. M....	42.6	32.2	17.2	19.3	18.5	20.3	93.0	95.2	13.8	15.1
10. S. W....	49.3	48.3	19.8	19.3	27.7	25.2	71.8	76.7	20.6	18.8
18. H. H....	48.3	37.6	19.3	20.5	20.6	21.9	94.1	93.5	15.3	18.4
29. L. F....	44.7	40.4	17.5	18.3	20.4	20.0	85.9	91.3	15.2	14.9
30. H. B....	48.4	37.5	18.4	18.9	20.0	22.1	91.9	85.3	14.9	16.5
31. C. P....	41.0	43.7	21.4	20.4	21.8	22.9	97.7	88.9	16.3	17.1

but one case (C. P.), the carbon dioxide content was less after exercise than immediately before. The oxygen content of the arterial blood increased following exercise in all but two cases (S. W. and C. P.) while the oxygen capacity showed a relatively smaller increase in most of the cases so that the oxygen saturation was increased following exercise in all but 3 cases. There was a marked decrease in the oxygen saturation following exercise in 2 cases and a slight fall in two others. All the other individuals responded as normal subjects, i.e., increase in the oxygen content and capacity with a slight rise in the oxygen saturation (Himwich and Barr (23)).

Observations of this nature in patients with chronic respiratory disease are not numerous. Himwich and Loebel (24) found in three patients with pulmonary emphysema that the oxygen con-

tent increased slightly after moderate exercise in two mild cases while in the third case there was marked reduction in the oxygen saturation as well as in its content. After exhausting exercise the saturation of hemoglobin may fall slightly even in normal subjects (24). Similar decrease was noted by Harrop (25) in a convalescent patient and also in three patients with polycythemia (Harrop and Heath (26)).

Numerous investigators have shown that almost invariably the hemoglobin and the red blood cells increase in both the venous and arterial blood following exercise, both in normal subjects and in patients with various diseases. The red blood cells, hematocrit and viscosity of the arterial blood was determined in 3 cases before and after exercise and in each case they were found to be increased following moderate effort.

#### DISCUSSION

From the results presented, it appears that in patients with cardiorespiratory disorders the degree of dyspnea during effort can be expressed fairly accurately in quantitative terms by means of the expression  $\frac{\text{total ventilation}}{\text{vital capacity}}$  and the pulmonary reserve. The factors responsible for increased ventilation at rest and for the appearance of dyspnea during moderate exertion in cases of heart disease have been amply discussed by Peabody and his associates (1), Harrison and his coworkers (2) and Christie and Meakins (3). It is our purpose to discuss similar factors responsible for the increase in total ventilation and the reduction in pulmonary reserve, which are usually associated with the appearance of breathlessness on moderate effort in patients with chronic pulmonary disease.

The greater increase in total ventilation in cardiac patients over the normal controls during moderate effort has been shown to be due in part to reflex stimulation of respiration by afferent impulses arising in the heart and lungs (2, 3). Although no direct measurements of the rigidity of the lung have been made in fibrosis, it is reasonable to suppose that, due to anatomical changes in this organ, there is decreased distensibility which may well cause increased sensitivity of these reflexes. Harrison and his coworkers (2) believed

that an initially high venous pressure and subsequent increase in this pressure during exercise in heart disease over that of the normal controls were important factors in the high total ventilation in such cases. In our series the venous pressure was elevated above the normal limits in 3 of the patients with heart disease, but it was not elevated above the limits of normal in any of the 5 cases of uncomplicated pneumokoniosis in which it was measured. Thus far there has been no evidence presented in the literature that would indicate the heart is demonstrably involved in pneumokoniosis which is uncomplicated by infection. Brooks (27), working in our laboratory, found that the cardiac output in patients with fibrosis was unaltered at rest and recently Miller (28) found that the blood velocity in such cases uncomplicated by heart failure was normal in contrast to those with heart disease where it was prolonged. Therefore, one is inclined to believe that the lungs rather than the heart are responsible for the reflex stimulation of respiration in cases of fibrosis. Several of the cases in this group had cardiac involvement and here additional factors are responsible for the increase in total ventilation. Not infrequently an extensive diffuse fibrosis with or without emphysema may give rise to hypertrophy of the right heart and later failure as a result of increased pressure in the lesser circuit due to a great reduction in the capillary bed of the lungs. Two of the patients (S. W. and J. B.) of this series fall into this category.

Factors such as increasing carbon dioxide tension and changes in acid base balance, in addition to those mentioned above, probably play a rôle in the production of dyspnea, but as Wiggers (29) wrote, "Until methods are devised which enable the experimenter to follow successive changes in carbon dioxide and alkali within shorter intervals of time, we may consider it probable that small transient variations are still being overlooked by the experimenter but not by the vigilant respiratory center." Another factor which seems of importance, which has been totally neglected, is the effect of cardiorespiratory disease on the mechanical efficiency of the human body as a machine, that is, on the ratio of energy converted into external work to total energy expenditure.

Aside from the above changes, the point of onset of dyspnea will vary in different individuals



according to the vital capacity. Peabody and his associates (1) have shown that the appearance of dyspnea is more closely related to the vital capacity than to the total ventilation. In cases of heart disease they found that dyspnea was proportional to the decrease in vital capacity. Hurtado et al. (17) drew similar conclusions in a study of patients with pulmonary fibrosis. The reduction of the vital capacity in the latter affection is probably due to the encroachment of fibrous tissue upon the functioning alveolar air spaces and by fixation of the diaphragm and ribs by pleural adhesions, thereby limiting maximum inspiration and expiration. Reduction in the vital capacity predisposes toward dyspnea, because it lowers the pulmonary reserve by reducing the maximum minute ventilation. The pulmonary reserve in normal individuals is great, and they can increase their resting ventilation by 9-fold. But in patients with cardio-respiratory disease, not only is the ventilation at rest higher but the minute volume during exertion reaches a higher level than in the normal controls. In addition, due to the reduction in the vital capacity, the highest ventilation that can be maintained is smaller than in the normal subjects. As a result of these alterations the pulmonary reserve in cases of chronic pulmonary disease is reduced and even moderate exertion produces the subjective sensation of dyspnea.

Patients with pulmonary emphysema have a great mechanical disadvantage in ventilating during effort. As a result of the loss of pulmonary elasticity, the intrapleural pressure fluctuates around that of the atmosphere, thereby decreasing the effectiveness of the diaphragm during inspiration. This is accompanied by an increase in the residual air, a reduction in the vital capacity and inefficient alveolar ventilation. The chest is already in the inspiratory position and the responsibility for any further increase in ventilation is placed upon the respiratory muscles. In consequence of the loss of elasticity of the lungs, patients with emphysema, in contrast to normal individuals, are unable to increase both the depth and rate of respiration. Either the depth is increased, accompanied by slower rate, or the rate is augmented at the expense of the depth. This is probably the most important factor in the prevention of hyperventilation on effort in such patients. This has been clearly demonstrated by the

results in this series of patients and substantiates the views of Christie (30).

An analysis of the changes in the arterial blood before and after exercise in cases of pulmonary fibrosis and emphysema throws further light on the pathological physiology of such disorders. Since most of the patients with fibrosis responded as do normal individuals in respect to oxygenation of the blood during moderate exertion, it appears that the amount of oxygen diffusing through the alveolar membrane is more than enough to compensate for the increasing rate of blood flow through the lungs. Although there are other factors that influence the saturation of the hemoglobin, Himwich and Barr (23) felt that they may be neglected for these two major ones. Of interest, however, are the cases of pulmonary emphysema and fibrosis which did not respond in the usual manner. These patients responded more like normal individuals performing exhaustive exercises. Under such conditions the saturation of the hemoglobin is decreased, and Himwich and Barr believed that under these circumstances the volume of blood flow may increase to an extent proportionately greater than the increase in oxygen diffusion.

Little is known concerning the rate of blood flow in chronic pulmonary disease during exertion. However, there are certain factors responsible for increasing the alveolar oxygen tension present in normal subjects during exercise that are lacking in patients with marked emphysema. Bohr (31) found that during exercise the residual air was increased in normal subjects. Our preliminary experiments substantiate his observations. Pulmonary distention of this nature will open more alveoli (32), will cause a stretching of the alveolar membrane resulting in a thinner and more permeable one (33), and will increase the ventilation coefficient, since the increase in tidal volume is much greater than the increase in the residual air. This increase in the alveolar air or so-called "mechanical buffer" tends to prevent a rapid fall of alveolar oxygen.

The benefit to be derived from these compensatory mechanisms must be almost negligible in emphysema. Although no measurements of the residual air have been made in emphysema during exertion, it is difficult to conceive how it can be significantly increased when the lungs and chest

at rest are already in an inspiratory position. Likewise, the relatively small increase in ventilation will not tend to increase the oxygen tension of the alveolar air.

Finally, the factors responsible for the decreased saturation of the hemoglobin after exercise in some cases of pulmonary fibrosis, may well be due to an impediment to the passage of oxygen through the alveolar membrane (Cases S. W. and H. B. had extensive fine reticular fibrosis throughout both lungs), either by thickening of the membrane or by a reduction in the area by the encroachment of fibrosis tissue. Another factor which may play a rôle is rapid and shallow breathing during moderate effort.

#### SUMMARY AND CONCLUSIONS

Measurements of the minute volume, the respiratory rate and the tidal volume were made in 20 normal individuals and 28 patients with chronic cardiac and pulmonary diseases during moderate and exhaustive effort. The ventilation equivalent for oxygen was determined at rest and during the fifth minute of moderate exertion. The results were correlated with the pulmonary capacity and its subdivisions, the ability to expand the chest and the oxygen saturation of the arterial blood. Blood was obtained from the radial artery immediately before and after moderately severe exercise in 9 cases of pulmonary fibrosis and emphysema.

From the results presented the following conclusions can be drawn.

1. Individuals with pulmonary fibrosis, rheumatic heart disease and obstructive emphysema have a greater minute ventilation and a more rapid and shallow type of breathing at rest and during moderate exertion than do normal individuals performing the same amount of muscular work. There are striking similarities between the patients with fibrosis and heart disease in their ventilatory response to exercise.

2. The degree of dyspnea is proportional to the expression  $\frac{\text{total ventilation}}{\text{vital capacity}}$ . Dyspnea is experienced when the value is greater than 51, and when this value is exceeded at low levels of work (300 kilogrammeters per minute for 5 minutes) it is an indication of pathological dyspnea. As the value for this expression increases, the vital ca-

capacity and the oxygen saturation of the arterial blood decreases and the ability to expand the chest diminishes.

3. The maximum minute ventilation that can be maintained for one and one-half minutes is only roughly proportional to the vital capacity in normal individuals, while in cases of chronic pulmonary disease the relation is closer. The maximum minute ventilation of an individual may be predicted from the observed vital capacity by assuming that the tidal volume will equal 41.4 per cent of the vital capacity and that the rate of breathing will be 35.5 per minute.

4. The pulmonary reserve is a measure of the tendency to dyspnea. Normal individuals can increase their resting minute volume 9-fold, while in patients who are disabled by pulmonary fibrosis and emphysema, the pulmonary reserve is so reduced that on moderate exertion the ventilation will constitute more than 60 per cent of the maximum minute ventilation.

5. The ventilation per 100 cc. oxygen absorbed increases from 2.40 liters for the normal subjects to 3.36 for the patients who complained of dyspnea on slight exertion. It is not a very good index of the degree of dyspnea, since there are marked irregularities in the individual values.

6. After exertion the oxygen content and the oxygen saturation of the arterial blood increases in the majority of the patients with pulmonary fibrosis. In several patients with marked emphysema and fibrosis the oxygen content and saturation were found to be decreased immediately after exercise.

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# A STUDY OF THE CARRIER CONDITION ASSOCIATED WITH TYPE II PNEUMONIA IN A CAMP OF THE CIVILIAN CONSERVATION CORPS<sup>1</sup>

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A veterans' camp of the Civilian Conservation Corps was opened in Madison County in New York State early in 1935; reforestation was the principal activity. The enrolled men were drawn in approximately equal numbers from civil life, from a veterans' camp in Vermont, and from one in New Jersey. A few came from a soldiers' home in a nearby county and a small number was transferred from other C.C.C. camps. Most of the men were between the ages of 37 and 49; the youngest was 32 and all but eight were under 50. The population had been in a continual state of flux. A large contingent from the camp in New Jersey arrived on November 21. From this date, however, until the investigation was concluded, on December 17, only one or two men entered.

During the latter part of October and early November, the men had been confined to barracks and to the recreation building, where they were in close contact throughout several rainy days. At this time, over half of them developed respiratory infections, which in many instances were persisting when the first investigation was undertaken on December 8.

The first case of pneumonia developed on November 9, in the course of this epidemic of colds, in a camper who had been transferred from the New Jersey camp eight days previously. He was treated in the camp infirmary, the sputum was not typed, and following an uneventful recovery, he was discharged from camp. It is noteworthy that no Type II pneumonia had previously occurred at this New Jersey camp. Between November 12 and December 1, six other cases of pneumonia developed in the Madison County camp, and still another occurred in a nearby vil-

lage; the individual in this instance was a bartender in a café which was a favorite rendezvous of the campers. All except the first patient were treated at the Cortland County Hospital where laboratory studies demonstrated the presence of Type II pneumococci. All of the patients recovered except one individual who, before the onset of his illness, suffered extreme exposure while under the influence of alcohol. Among those who recovered, Type II pneumococci were isolated once from the blood of one individual and on two separate occasions from that of another. The testing of a strain obtained from the latter patient, which was maintained in semisolid medium, revealed that it was highly virulent for mice; that is, mice weighing between sixteen and twenty-two grams, inoculated in duplicate with 0.0000001 cc. of an 18-hour broth culture died within forty-eight hours. None of the patients received antipneumococcus serum.

On December 8, 1935, an investigation was undertaken to determine the incidence and distribution of carriers of Type II pneumococci within the camp. Throats of the campers were cultured, and pertinent data were secured through the taking of individual histories.

Cultures were obtained at the rate of from fifteen to thirty a day, usually just before mess when the men were congregated in their respective barracks; care was taken in swabbing out the tonsillar crypts and in reaching as high in the nasopharynx as a straight swab would allow. The swabs were then placed immediately in Avery's broth, and were incubated for from four to seven hours at 37.5° C.; of the resulting growth, from 0.3 to 0.5 cc., depending on the density, were inoculated into mice. The peritoneal exudates of those mice which died spontaneously were examined directly for the presence of Type II pneumococci by the Neufeld technic; the heart's blood, in each instance, was streaked on blood-agar plates, and after twenty-four hours' incubation, the resulting growth was examined directly by the

<sup>1</sup> Presented at the meeting of the New York State Association of Public Health Laboratories, Poughkeepsie, New York, May 25, 1936.

Neufeld method.<sup>2</sup> Most of the strains of pneumococcus Type II were then transferred to blood-agar slants, for confirmatory tests by the macroscopic tube agglutination method. Mice which failed to develop symptoms of illness within forty-eight hours were not examined. During the final survey in April, however, such animals were chloroformed and autopsied; in rare instances, pneumococci were isolated in this manner, but the types were of minor interest.

Among 142 individuals examined in the five barracks, twenty-seven were found to be carriers, an incidence of 19 per cent. Table I indicates

TABLE I

*Distribution of cases of Type II pneumonia and carriers of Type II pneumococci*

Barrack number	Number of men in barrack at time of survey *	Number of cases	Number of carriers
I	34	2	5
II	31	2	4
III	30	1	6
IV	28	1	4
V	26	1	8

\* All but seven men were studied.

the relatively even distribution among the five barracks of the cases of Type II pneumonia and of individuals carrying pneumococci of that type in the throat.

Among seven officers examined, who were living in the officers' quarters, two whose special duties brought them into intimate contact with the enrolled men were found to be carriers. No carriers were discovered among nine foresters living in the forestry quarters or among six men on special duty scattered in various other buildings. Only one of the three men in the infirmary convalescing from Type II pneumonia proved to be a carrier. Two healthy bartenders at the café mentioned previously were not found to be carriers. The relationships between the carrier state and upper respiratory infection as well as between the carrier state and history of contact were not striking. Of the carriers, 30 per cent were suffering from some form of upper respiratory infection at the time of the investigation, 40 per cent gave a history of a recent cold, and 30 per

cent gave no such history; 25 per cent of the carriers recalled intimate contact with one or more of the patients having pneumonia immediately prior to or during the illness. Of the noncarriers, 21 per cent were suffering from upper respiratory infection, 31 per cent gave a history of a recent cold, and 48 per cent gave no such history; 16 per cent of the noncarriers recalled contact with one or more of the pneumonia patients. It was possible only in rare instances to formulate a definite idea as to the source of the Type II pneumococci in a given case or carrier, since the opportunity for chance contact in the mess hall and general assembly room was great.

An incident of considerable interest was the subsequent development of a case of Type II pneumonia in barrack Number IV, on December 21, three weeks after the onset of the next preceding case, and only six days after the man's throat had been cultured. He had not been found to be a carrier of Type II pneumococci, but of Type XXI; however, his bed was next to that of a Type II carrier.

In order to secure information relative to the persistence of the carrier condition, a second survey was undertaken. The camp in Madison County was disbanded early in 1936, and most of the men were transferred to other companies in two camps located in Orange County, where they were engaged in flood control. The nature of their work entailed considerable exposure, which might be expected to favor the development of respiratory infections. However, no such epidemic had occurred since the beginning of the year. On February 26, twenty of the original carriers and three of the men who had recovered from Type II pneumonia were again examined to determine whether or not a sufficiently large number of them were still harboring the Type II pneumococci to warrant making a general survey. From the throats of nine of the original carriers and two of the recovered cases, Type II pneumococci were isolated. It was learned at this time that although a few sporadic cases of pneumonia had occurred in these camps, none of them had been incited by Type II pneumococci. In view of the possibility that these strains of Type II pneumococci might be of comparatively low virulence, tests were performed with the eleven strains isolated at this time from the heart's blood of each

<sup>2</sup> The pneumococcus typing and virulence tests during these surveys were undertaken by Miss Florence M. Varley.

mouse which had been inoculated with Avery's broth cultures, and stored at 8° C. for twelve days in serum semisolid medium (1). All of the strains were found to be highly virulent; mice inoculated in duplicate with 0.00000001 cc. of a 14-hour broth culture, died within forty-eight hours; pour plates of this dilution indicated that an average of five microorganisms had been injected.

In conjunction with these studies of the virulence of the eleven strains of Type II pneumococci, the factor of individual host resistance was investigated.<sup>3</sup> With the blood serum which had been obtained from the three recovered cases and from all but one of the twenty original<sup>4</sup> carriers who were studied at this time, mouse protection tests were performed against pneumococcus Type II. With sera of selected individuals in this group, similar tests were made using pneumococcus Type I. Twenty of these sera were also tested for agglutination and precipitation. For purposes of control, the sera of ten normal men, who gave no history of pneumonia, who were of approximately the same age as the campers, but who had no connection with the camps, were similarly tested. Throat cultures obtained from this group of control individuals, at the time the blood was collected, failed to reveal the presence of pneumococci Types I or II.

In the mouse protection tests, 0.1 cc. of serum was tested with different doses of a 16-hour culture of the standard strains, Type II (Number 53) and Type I (Number 1). The dilutions of culture and of serum were prepared so that the required amount of each was contained in 0.5 cc. The 0.5 cc. of diluted culture and the 0.5 cc. of diluted serum were thoroughly mixed in the barrel of the syringe and immediately injected into the peritoneum of a mouse weighing from eighteen to twenty-two grams. Two dilutions of culture were used in each test, and three mice for each dilution. Those

<sup>3</sup> These serological tests were performed at the central laboratory in Albany under the supervision of Miss Jessie L. Hendry.

<sup>4</sup> "Original carriers" refers to those men who were shown to be carriers of Type II pneumococci during the December survey.

"Persistent carriers" refers to those original carriers who were again shown to be carriers of Type II pneumococci in February.

"Former carriers" refers to those original carriers who, by February, had apparently ceased to be carriers of Type II pneumococci.

mice surviving for ninety-six hours or longer were considered protected by the serum. Heart's blood cultures were made at autopsy. Determinations of the minimal fatal dose (M.F.D.) made with each test according to the method described in a recent paper by Kirkbride, Hendry, and Murdiek (2) indicated that the injection of one microorganism was sufficient to kill. In most instances, the results presented were obtained in two tests; in the others, three or only one was necessary.

While all of the twenty-two sera were tested against the Type II standard culture, only thirteen were tested with the Type I culture; these thirteen included sera from five men who had been inoculated in 1934 with Type I and Type II soluble antigen (an active form of the polysaccharide of Heidelberger and Avery) as a prophylactic measure against pneumonia of these types (3); of the five, three were persistent carriers and two were former carriers. The ten control sera were tested against both the Type I and Type II strains.

The results of these protection tests are summarized in Table II. The serum from only one of the three cases which had recovered from Type II pneumonia protected against as much as 100 M.F.D. of the Type II culture. On the other hand, sera from seven of the eleven former carriers and from five of the eight persistent carriers protected against 100 or more M.F.D. of the Type II culture; among the sera showing a significant degree of protective activity, those from two former and two persistent carriers, none of whom had received the immunizing dose, protected against as much as 10,000 M.F.D.; indeed, the sera from only three of the five who had received the Felton antigens protected against as much as 100 M.F.D. Only two of the ten control sera protected against as much as 100 M.F.D. In corresponding tests with the Type I culture, the serum from not one of the three recovered cases and from only one of the five carriers, none of whom had received the Felton antigens, afforded protection against as much as 100 M.F.D. In contrast, the sera from three of the five carriers who had received the Felton antigens protected against 100 or more M.F.D., and of these three, one protected against 10,000 M.F.D. Only two of the ten control sera protected against as much as 100 M.F.D.

All but two of the sera were of sufficient amount to permit agglutination tests in which cell

TABLE II

*Results of potency tests of sera obtained February 26, 1936, from original carriers*

Condition	Number tested		Individual sera which, in 0.1 cc. amounts, protected against the following doses of Type II pneumococcus culture, strain Number 53						Number tested		Individual sera which, in 0.1 cc. amounts, protected against the following doses of Type I pneumococcus culture, strain Number 1					
			1 or -1 M.F.D.		100 M.F.D.		10,000 M.F.D.				1 or -1 M.F.D.		100 M.F.D.		10,000 M.F.D.	
Recovered cases	3		2		1*				3		3*					
Persistent carriers	F.A. 3	5	F.A. 1	2	F.A. 2	1	F.A. 2	2	F.A. 3	3	F.A. 2	2	F.A. 1	1	F.A.	
Former carriers	2	9	1†	3	1‡	4		2	2	2		2	1‡		1†	
Total sera of carriers studied	5	14	2	5	3	5		4	5	5	2	4	2	1	1	
	19		7		8		4		10		6		3		1	
Controls	10		8		2§				10		8		2			

F.A. = Received Felton Type I and Type II antigens in 1934.

\* Undiluted serum of one person agglutinated the Type II cells and precipitated the supernatant broth culture.

† Skin reaction elicited in this person with Type I polysaccharide, March 31, 1936.

‡ Undiluted serum of this person agglutinated Type I cells. Skin reaction elicited with Type I polysaccharide, March 31, 1936.

§ One probably less than 100 M.F.D.

suspensions of the standard Type I and Type II strains were used, and also precipitation tests in which the supernatant fluids from the broth cultures were employed (4). The sera were tested both undiluted and diluted 1:5. Reactions were obtained with only two of these sera, and with none of the control sera; the undiluted serum from one of the recovered cases agglutinated the Type II cells and precipitated the supernatant fluid;<sup>5</sup> the undiluted serum from one former carrier who had received the Felton antigens, agglutinated only the Type I cells.

As a result of the findings obtained during the second survey, a third and final one was made beginning March 31. On this occasion, skin tests were performed according to the method of Tillett and Francis (5), employing pneumococcus type-specific polysaccharides I and II, prepared by Dr. Rachel Brown of this laboratory. Eleven available members of the group of twenty-two whose blood sera were studied during the preceding survey were selected for the tests. None of the men showed any appreciable skin reaction to the Type II polysaccharide, but of four original carriers in

this group who had received the Felton antigens, two developed marked skin reactions within thirty minutes after the injection of the Type I polysaccharide, characterized in one case by wheal and erythema, and in the other, by a large area of erythema alone.

After the completion of the skin testing, the third survey of pneumococcus carriers was undertaken. A certain company in the smaller of these two camps in Orange County was chosen for study since the largest number of persistent carriers had been assigned to this company, and also because of the fact that a severe epidemic of upper respiratory infection had occurred among the men during the early part of March, at which time a number of them required hospitalization in the infirmary. Of the eight original carriers and two former cases assigned to this particular company, among whom four carriers and one case had been found still to harbor Type II pneumococci at the time of the survey in February, all but one were still available for study. Of the four carriers from whose throats Type II pneumococci were isolated in December and again in February, three were found to be carriers of this microorganism in April. None of the other four original carriers could be shown to have reverted to the carrier

<sup>5</sup> Unfortunately this individual was not available for skin testing in the subsequent investigation.



state; one of the two former cases still available for study had apparently ceased to be a carrier since the previous examination.

The throats of seventy other men in the company were cultured. This number represented practically all who were remaining in preparation for evacuating the camp in the middle of April. Eighteen were men transferred from the camp in Madison County who during the survey there in December were not found to be carriers of Type II pneumococci. Most of the others had been transferred in October, 1935, from a camp in Montgomery County where the men were said to have been remarkably free from respiratory infections.

Strains of pneumococcus Type II were isolated from the throats of only two of the seventy men studied. Neither gave a history of contact with patients having pneumonia, and neither had been in the camp in Madison County. Both were housed in a barrack in which none of the proven carriers were lodged. The inference is fairly strong, although by no means conclusive, that these two men developed the carrier state through association with the carriers in the mess hall, in the recreation hall, or in the field. The throats of these two men were recultured two days later, and virulence tests were performed with the two strains of Type II pneumococci isolated from the heart's blood of each inoculated mouse. Furthermore, because of the possibility that the virulence of the strains might have been raised by passage through mice, successful attempts were made in these two instances to isolate Type II pneumococci from blood-agar plates streaked with Avery's broth culture. While in one instance the strain was isolated with considerable difficulty, in the other, no trouble was encountered, for examination of Avery's broth culture by the Neufeld technic indicated that an almost pure culture of Type II pneumococci was present. Strains isolated from the heart's blood of the two mice and directly from the blood-agar plates all proved to be highly virulent; 0.00000001 cc. of a 12-hour broth culture, injected into mice in duplicate, killed them within forty-eight hours; pour plates of this dilution indicated that an average of two microorganisms had been injected.

The relationship between the carrier state and upper respiratory infection in this survey in April

was no more evident than in the December survey. Of the three persistent carriers, one was suffering at this time from a head cold; none gave a history of recent respiratory infection. Of the two carriers who were detected for the first time during the April survey, one gave a history of a slight throat irritation some weeks previously, while the other, from whose throat Type II pneumococci were isolated in large numbers, gave a negative history. Of the noncarriers, 14 per cent were suffering from upper respiratory infection; 36 per cent gave a history of a recent cold; and 50 per cent gave no such history.

Although a general survey of the larger of these two camps seemed impractical, four of the men who were found to be carriers both in December and in February were again examined; Type II pneumococci were isolated from the throats of three of them; all four gave a history of very recent respiratory infection.

Quite apart from the main purpose of the investigation, some of the strains of pneumococci isolated from the throats of men who were not found to be carriers of Type II were also studied. Less attention was paid to this feature during the first survey than during the last two when the types of all strains of pneumococci isolated from the heart's blood or peritoneal exudate of each inoculated mouse were identified. Eighty-four, sixteen, and fifty-four strains of pneumococci were isolated and typed during the three successive investigations respectively.

Table III is a recapitulation of the incidence of various types of pneumococci exclusive of strains of pneumococcus Type II isolated from recovered cases. In only six instances was the same type of pneumococcus isolated on more than one occasion from the same individual. Pneumococci Types I, V, VII, and XIV, which frequently incite pneumonia, were not encountered during any of these surveys. On the other hand, Types III and VIII, both important pathogenic agents, were not infrequently observed.

#### LITERATURE

Reports in the literature indicate that the occurrence of Type II pneumococci in a normal throat is unusual. Stillman (6) in 1917, examined the saliva of 297 normal individuals, and found pneumococci in 116 instances; pneumococ-



TABLE III

*Types of pneumococci found in throat cultures (excluding strains of Type II from recovered cases)*

Pneumococcus type	December	February	April
I.....			
II.....	29	9	8
III.....	13	1	10
IV.....	4	3	7
V.....			
VI.....	6	1	2
VII.....			
VIII.....	4	1	4
IX.....	1		
X.....	1		2
XI.....	2		6
XII.....			
XIII.....	1		2
XIV.....			
XV.....	1		1
XVI.....	1		1
XVII.....	1	1	
XVIII.....	5		6
XIX.....	1		
XX.....	3		
XXI.....	1		
XXII.....	2		1
XXIII.....	2		
XXIV.....	2		1
XXV.....			
XXVI.....	1		1
XXVII.....			
XXVIII.....	1		1
XXIX.....	2		1
XXX.....			
XXXI.....			
XXXII.....			
Total numbers of pneumococcus strains, isolated and typed.....	84	16	54

cus Type I was observed in one specimen, and Type II not at all. More recently, Webster and Hughes (7) found pneumococci in about 80 per cent of the 105 healthy adults and children studied. In a total of more than 3,000 cultures, Type II pneumococci were found in but three single cultures from two individuals and Type I was obtained on only one occasion from a single individual.

In order to determine the frequency with which normal individuals in contact with cases of Type I or Type II pneumonia acquire these types of pneumococci, Stillman (6) dispatched a nurse to the home as soon as possible after a patient suffering from pneumonia due to Type I or Type II pneumococcus was admitted to the Rockefeller Hospital. Specimens of saliva were collected from all persons who had come in contact with the patient. Of 107 contacts in a total of twenty-

eight households where cases of Type I pneumonia had occurred, 15 per cent were found to harbor Type I pneumococci; of seventy-seven contacts in a total of twenty-four households where cases of Type II pneumonia had occurred, 6 per cent were found to harbor Type II pneumococci. The average carrying period for Type I was twenty-five days, and for Type II, forty-three days.

Cole (8) states that "in most instances in which pneumococci of Type I or Type II have been found in the mouths of normal persons, these persons have very recently been closely associated with patients suffering from pneumonia due to the same type of pneumococcus as that isolated" and again (9) "One of the most interesting facts which has appeared as a result of the study of the different types of pneumococci is that pneumococci of the so-called fixed types, I and II, which are responsible for such a large proportion of the cases of pneumonia, are not found in normal mouths, and that within a relatively short time after convalescence they disappear from the mouths of those who have suffered from pneumonia due to one or other of them."

In a study of the epidemiology of pneumonia, Smillie (10) examined strains of pneumococci in the nasopharynx of immediate family contacts, and concluded from his series of 582 contacts and 493 controls that Types I and II were much more prevalent in the nasopharynges of contacts than in the population at large. This finding did not prove to be true with regard to many of the types of pneumococci which were formerly included in Group IV.

The epidemic nature of lobar pneumonia and the probable rôle of the carrier has been discussed by Gundel and Wallbruch (11, 12) in a survey of a small village of three hundred inhabitants near Berlin, where an epidemic of Type I pneumonia had occurred during February, 1935. This outbreak followed two weeks after an epidemic of "grippe"; the first case developed in the village school, where the disease spread rapidly to other pupils and then among their respective families. These authors have convincingly traced the spread of the disease from one individual to the next, through the medium of cases and carriers, of which there were a large number

among the examined contacts. On the basis of these findings and of the few similar reports in the literature, these authors emphasize that lobar pneumonia should be classed as a true contagious disease, which may, under certain circumstances, become epidemic.

With regard to the serological studies in connection with the carrier condition, Tilghman and Finland (13) have reported suggestive findings in tests performed with the blood of individuals in contact with cases of pneumonia, comparable to the results described in this paper. They have shown that specific antibodies may develop in individuals who are carriers of the disease-producing pneumococcus but who do not become ill with the infection.

#### SUMMARY AND CONCLUSIONS

An epidemic of respiratory infection in a veterans' camp of the Civilian Conservation Corps, located in Madison County in New York State, was followed by an outbreak of lobar pneumonia, in which the mortality rate was low; only one of nine cases died (Type II pneumococci were isolated from the blood of two of the patients who recovered).

After the outbreak of pneumonia, an investigation was undertaken to determine the incidence and distribution of carriers of Type II pneumococci within the camp. Nineteen per cent of the enrolled men living in the barracks were found to be carriers of this microorganism.

The last case of Type II pneumonia at this camp developed three weeks after the next preceding one. This lapse of time, together with the failure to demonstrate the presence of Type II pneumococci in the throat only six days before the onset of pneumonia, lends strong support to the belief that the infection was acquired in this instance not from one of the cases but rather through contact with a carrier, not unlikely the carrier in the adjoining bed.

Ten weeks after the conclusion of the first investigation, a second was undertaken in camps in Orange County to which most of the campers had been transferred. The carrier state was found to persist in a number of the original carriers. Tests with the isolated strains of Type II pneumococci revealed a high degree of virulence

for mice. Serological tests with the blood of carriers suggested that in certain instances there may have been a significant correlation between the protective activity of individual blood serum against the Type II pneumococcus and the presence or recent existence of Type II pneumococci in the throat. Protective activity against the Type I pneumococcus was, on the whole, more evident in the group of sera from those who had received the Felton Type I and Type II antigen in 1934, than in the sera from those who had not; on the other hand, such evidence was lacking with regard to protection against Type II. The small number of sera examined prevents the drawing of definite conclusions. Skin tests, performed five weeks later, with polysaccharides Types I and II yielded reactions to Type I in only two of the group treated by Felton.

A final survey was undertaken fifteen weeks after the conclusion of the first survey. A number of the original carriers were found still to harbor Type II pneumococci in the throat, and two men who, as far as is known had come in contact with no case of Type II pneumonia, were shown in this survey to be carriers of virulent Type II pneumococci, thus suggesting that the carrier state in these instances had developed through contact with other carriers. In view of the persistence and the apparent spread of the Type II carrier condition, it is noteworthy that although an epidemic of respiratory infection seemed to have prepared the way for an outbreak of Type II pneumonia in the Madison County camp, a similar epidemic in the camp in Orange County was followed by no cases of Type II pneumonia.

These investigations lend support to the accumulating evidence that the carrier is an important factor in the transmission of pneumococcus infection. On the other hand, the question of why certain individuals develop pneumonia on exposure to virulent pneumococci while others become carriers, can not be adequately explained on the basis of the studies of host resistance.

We are indebted to Dr. William A. Wall, who provided the facilities of his laboratory during the first study, and to the medical officers of the camps: Lieutenant Colonel Blank, Lieutenants Stein, Troupin, and Freeman.

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# THE EFFECT ON THE KIDNEY OF BILATERAL SPLANCHNICECTOMY IN PATIENTS WITH HYPERTENSION

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Recently there has been a renewed interest in the etiology and treatment of hypertension (hypertensive vascular disease) and its relationship to the kidneys. A surgical method of treatment of hypertension which was devised by Peet (1) consists of a bilateral resectioning of the major and minor splanchnic nerves and of the lower dorsal sympathetic chain including the 10th, 11th and 12th ganglia, supradiaphragmatically. This procedure has been employed in a sufficient number of cases so that the trend of results is indicated. It is the purpose of this communication to report the effect of this operation upon the kidneys, as it is shown by measurement of renal function and urinary abnormalities, and to correlate this effect with the blood pressure changes. Considerations of the rationale behind this treatment of hypertension, selection of candidates, technical procedure, and results other than the renal effect will not be discussed.

## METHOD OF STUDY

Every candidate for this operation was carefully studied in order to exclude all cases having hypertension secondary to known organic disease. Special care was taken to exclude those patients with hemorrhagic (glomerular) nephritis having elevated blood pressure. As a result only cases considered to have primary ("arterial") hypertension (hypertensive vascular disease), were treated by splanchnicectomy. This report includes observations on only those patients whom we have studied over a period of three months, or longer, after operation.

Before, and at intervals after this operation, blood pressure and renal function were measured, and the urine was analyzed. The blood pressure was in every case measured with a mercury manometer several times while the patient was lying down. Renal function was measured by the urea clearance test of Van Slyke and Cope

(2) and by the Lashmet-Newburgh concentration test (3). Proteinuria was measured by sulphosalicylic acid precipitation (4), and the formed elements in the urinary sediment were counted by the Addis method (5).

*Criteria for classification.* We have adopted the normal values found by the originators of the tests of renal function, i.e., for the concentration test a nonprotein specific gravity of the urine of 1.029 or more, and for the urea clearance, values between 75 and 125 per cent of the mean normal. The renal function was considered to be impaired when the result of either of the functional tests was below normal. A significant change in renal function was considered to consist of a change in specific gravity of 0.003 or more, or a change of 15 per cent or more in urea clearance.

It is always difficult to discuss changes in blood pressure because of the fluctuations encountered. For the purpose of classification, we have grouped the patients according to the change in blood pressure following splanchnicectomy as follows: 1, those whose blood pressure was reduced to 160/100 mm. Hg, or less; 2, those whose blood pressures did not remain below 160/100, but whose systolic blood pressure was reduced more than 60 mm. Hg, or whose diastolic pressure was reduced more than 30 mm.; 3, those whose systolic blood pressure was reduced from 30 to 60 mm., or whose diastolic pressure was reduced 15 to 30 mm. Hg.

## RESULTS

By this method we have now studied forty-eight cases. The data on these cases are presented in Table I. A summary of the relationship between the renal status and the blood pressure will be found in Table II.

In almost every case there was a sharp fall in blood pressure to normal or below, immediately after splanchnicectomy. It then followed one of several courses: in some it remained approx-

TABLE I

Data on each of the 48 cases studied, showing pre- and postoperative renal activity and blood pressure

Case number and initials	Sex	Age at time of operation	Known duration of hypertension	Time of observation	Blood pressure	Concentrating ability of kidneys	Urea clearance	Urine		Classification in Table II	Remarks
								Proteinuria	Hematuria		
		years			mm. Hg	nonprotein specific gravity	per cent of mean normal	per cent	millions of r.b.c.'s per 12 hours		
1 W. L.	M	29	9 mos.	Pre-op. 2 wks. postop. 6 wks. postop.	236/166 134/90 140-160	1.014 1.022 1.026	106 58 80	0.3 Trace 0	0 0 0	1Ab	
				5 mos. postop. 11 mos. postop. 15 mos. postop. 29 mos. postop.	100-116 138/90 150/90 156/100	1.033 1.033 1.031	108 125 95	0 0 0	0 0 0		
2 M. P.	F	41	5 yrs.	Pre-op. 3 wks. postop. 3 mos. postop.	205-240 125-130 120/80	1.024	59	Trace	0	1Ab	
				4½ mos. postop.	140-160 97-100 146/94	1.030	91 79	0 0	0 0		
3 J. J.	M	45	1½ yrs.	Pre-op. 3 wks. postop. 6 mos. postop.	190-230 100-150 148/96	1.028	71	0	0	1Ab	
					142-160 98-100	1.031	75	0	0		
4 V. E.	F	22	3 yrs.	Pre-op. 2 wks. postop. 2 mos. postop. 5 mos. postop. 8 mos. postop. 11 mos. postop.	280/190 150/110 146/102 150/120 136/90 132/95	1.033 1.025 1.028 1.029	87 38 82 85	0.12 Trace Sl. tr. Sl. tr.	2.2 0 0 0	1Aa	
5 P. D.	M	46	15 yrs.	Pre-op. 1 mo. postop. 4 mos. postop.	180-190 110-120 140/90 140/88	1.028 1.0255	91 92	0 0	0 0	1Ac	Bladder residual at time of concentration test
6 G. P.	F	23	2 yrs.	Pre-op. 2 wks. postop. 4 mos. postop.	248-270 138 180/120 160/100	1.026 1.026	80	Trace 0	0 0	1Ac	
7 C. L.	M	35	7 mos.	Pre-op. 2 wks. postop. 2½ mos. postop. 6 mos. postop. 9 mos. postop. 16 mos. postop.	268/176 180/130 200/128 190/130 180/125 180/140	1.019 1.020 1.030 1.030 1.030	40 95 113 85 125 98	0.3 0.05 Trace Trace 0 0	280 0 0 0 0 0	1Bb	
8 S. B.	M	53	4 yrs.	Pre-op. 3 wks. postop. 5 mos. postop. 8 mos. postop. 10 mos. postop. 12 mos. postop. 14 mos. postop. 17 mos. postop.	230-260 170-180 125-130 90 160-166 109 160/110 150-165 105-110 145/100 150 105-110 148/110	1.023 1.022 1.027 1.026 1.028 1.030	70 51 77 83 89	0.1 Trace Trace Trace 0 0 0	3.7 0 0 0 0 0 0	1Bb	

# SPLANCHNICECTOMY IN HYPERTENSION

TABLE I—Continued

Case number and initials	Sex	Age at time of operation	Known duration of hypertension	Time of observation	Blood pressure	Concentrating ability of kidneys	Urea clearance	Urine		Classification in Table II	Remarks
								Proteinuria	Hematuria		
		years			mm. Hg	nonprotein specific gravity	per cent of mean normal	per cent	millions of r.b.c.'s per 12 hours		
9 E. G.	F	46	3 yrs.	Pre-op. 2 wks. postop. 6 mos. postop.	250/134 212/100 150/100	1.024  1.034	72  136	0.05  0	0  1.25	1Bb	
10 M. W.	F	44	3 yrs.	Pre-op. 2 wks. postop. 3 mos. postop. 6 mos. postop.	250-260 150-162 140/95 178-220 110-120 186-200 112	1.023  1.027 1.028	77  86 111	Trace 0 0	0 0 0	1Bb	
11 E. H.	F	23	6 yrs.	Pre-op. 2 wks. postop. 2 mos. postop. 6 mos. postop.	230-240 140-160 180/130 176/128 170/128	1.026  1.030 1.033	84  88 89	Trace Trace Trace	0 0 0	1Bb	
12 O. Mc.	M	44	6 mos.	Pre-op. 2 wks. postop. 2 mos. postop. 3 mos. postop. 6 mos. postop.	224-256 140-172 170 110-116 150/110 170/116 190/124	1.021  1.028 1.024	  84 97 120	Trace Trace Sl. tr.	5 0 1.3	1Bb	
13 A. B.	F	40	6 yrs.	Pre-op. 1 mo. postop. 6 mos. postop.	244-276 148-156 160/120 178/112	1.036  103	97  103	Trace 0	0 0	1Ba	
14 H. R.	F	49	2 yrs.	Pre-op. 3 wks. postop. 5 mos. postop. 9 mos. postop.	210-252 120-148 160/100 155/100 174/105	1.030  1.032 1.029	101  0 0	Trace 0 0	0 0 0	1Ba	
15 F. M.	M	38	1 yr.	Pre-op. 2 wks. postop. 5 mos. postop.	240-260 145-150 210/115 210 120-130	1.025  1.027 1.029	71  72 86	0.4 0.1 Trace	0 0 0	1Cb	
16 L. M.	M	31	4 yrs.	Pre-op. 2 wks. postop. 2 mos. postop. 6 mos. postop. 16 mos. postop.	220-226 145-158 140-150 90-100 190/140 180-200 134-140	1.024  1.026 1.026	56  72 60 92	Trace Trace Trace	0 0 0	1Cb	Acute coryza
17 S. O.	F	33	?	Pre-op. 3 wks. postop. 6 mos. postop.	210 130-140 130-160 70-110 170-198 110-126	1.026  1.029	59  81	Trace Trace	0	1Cb	

TABLE I—Continued

Case number and initials	Sex	Age at time of operation	Known duration of hypertension	Time of observation	Blood pressure	Concentrating ability of kidneys	Urea clearance	Urine		Classification in Table II	Remarks
								Proteinuria	Hematuria		
		years			mm. Hg	nonprotein specific gravity	per cent of mean normal	per cent	millions of r.b.c.'s per 12 hours		
18 M. S.	F	33	2 yrs.	Pre-op.	190-220	1.028	76	Trace	0	1Cb	
				3 wks. postop. 6 mos. postop.	94-130 154/116 140-220 84-108	1.033	92	Trace	0		
19 N. M.	F	39	3 yrs.	Pre-op.	198-260	1.016	56	2.0	Gross	1Cb	
				2 wks. postop. 4 mos. postop.	138 200/130 180-220 130	1.023	109	Trace	0		
20 H. F.	M	46	2 yrs.	Pre-op.	235-280	1.029	89	Trace	0	1Ca	
				3 wks. postop. 8 mos. postop.	126-170 195/130 220-240 149	1.029	78	Trace	0		
21 D. P.	M	41	7 yrs.	Pre-op.	234-250	1.025	88	Trace	0	1Cc	
				1 mo. postop. 10 mos. postop.	150-160 210/105 210/136	1.024	100	0	0		
22 E. C.	F	45	1½ yrs.	Pre-op.	198-244	1.026	96	0.1		1Cc	
				2 wks. postop.	114-140						
				3 mos. postop.	132/82 168/230	1.027	108	0			
23 L. V.	F	33	4 yrs.	6 mos. postop.	100-130 180/110	1.028	122	Sl. tr.		1Cc	? pituitary disease with diabetes insipidus
				4 yrs. pre-op. 3 yrs. pre-op. 2 yrs. pre-op. 1 wk. pre-op. 3 wks. postop. 10 mos. postop.	158/100 158/112 182/130 202/132 130/90 160 112-120	1.018 1.016 1.012 1.012	  129 92	0 Trace Trace Trace	  0 0		
24 L. B.	M	45	5 yrs.	Pre-op.	210-220	1.030	71	Sl. tr.		1Cd	
				3 wks. postop. 14 mos. postop.	120 150/95 180-190 110-130	1.025		Trace			
25 H. H.	F	44	3 yrs.	Pre-op.	240-248	1.033	75	Trace	0	2a	
				3 wks. postop.	154-165 200-240						
				4 mos. postop. 6 mos. postop.	140 235/130 226-250	1.029 1.032	76	Trace Trace	0 0		
				21 mos. postop.	132-142 242/148	1.034	80	Trace	0		
26 H. K.	M	37	3 yrs.	Pre-op.	190-226		80	Trace		2a	
				3 wks. postop. 8 mos. postop.	130-160 180/110 230/150		84	Trace			

TABLE 1—Continued

Case number and initials	Sex	Age at time of operation	Known duration of hypertension	Time of observation	Blood pressure	Concentrating ability of kidneys	Urea clearance	Urine		Classification in Table II	Remarks
								Proteinuria	Hematuria		
27 H. S.	M	36	8 mos.	Pre-op.	198-215	1.018	91	0	0	2d	
				3 mos. postop.	120-148 168-180	1.025	66	0.05	0		
				4 mos. postop.	120 186/110		74	Trace	0		
				6 mos. postop.	204/132						
				13 mos. postop.	200/128	1.024		Trace	0		
				24 mos. postop.	194-204 120-126						
28 T. A.	M	47	6 mos.	Pre-op.	185-204	1.017	83	Trace	0	2d	
				3 wks. postop.	135 170/140						
				9 mos. postop.	190/130	1.030	125	0	2.8		
29 F. N.	F	39	3 yrs.	Pre-op.	220-250	1.022	26	Trace	0	2b	
				3 wks. postop.	130-150 192/110						
				3 mos. postop.	220/130	1.023	35	Trace	0		
				6 mos. postop.	240/140	1.021	39	Trace	0		
				12 mos. postop.	244/140	1.020		0.1	0		
				21 mos. postop.	230-250 152	1.021	39	Trace	0		
30 W. Mc.	M	55	4 yrs.	Pre-op.	188-226	1.025		Trace		2b	
				5 wks. postop.	120-135 170-198	1.029	67	Trace			
				5 mos. postop.	122-128 195/120	1.027	75	Trace			
				12 mos. postop.	185-220 120-140	1.025		Trace			
31 G. P.	M	42	5 yrs.	Pre-op.	210-252	1.021	58	0.5	53	2b	
				3 wks. postop.	150 191/120						
				5 mos. postop.	210/144	1.021	62	0.1	4.0		
32 H. B.	F	43	8 yrs.	Pre-op.	200-245	1.025	47	0.1	3.0	2b	
				2 wks. postop.	130-145 220/120						
				3 mos. postop.	180/100	1.024	54	Trace	1.4		
				7 mos. postop.	240-260	1.023	60	Sl. tr.	0		
					150						
33 M. S.	F	42	5 yrs.	Pre-op.	210-222	1.027	92	Trace		2b	
				3 wks. postop.	130-134 190/112						
				10 mos. postop.	180-210 130-140	1.026	102	Trace			
34 R. H.	F	44	4 yrs.	Pre-op.	208-250	1.021	112	Trace		2b	
				3 wks. postop.	126-136 200/120						
				14 mos. postop.	240/144		77	Trace			



TABLE I—Continued

Case number and initials	Sex	Age at time of operation	Known duration of hypertension	Time of observation	Blood pressure	Concentrating ability of kidneys	Urea clearance	Urine		Classification in Table II	Remarks
								Proteinuria	Hematuria		
		years			mm. Hg	nonprotein specific gravity	per cent of mean normal	per cent	millions of r.b.c.'s per 12 hours		
35 A. V.	F	48	5 yrs.	Pre-op.	242-300	1.017	21	0.25			
				1 mo. postop.	160-170 180-210	1.019	26	Trace		2b	
				3 mos. postop.	120 230-240	1.016	24	Trace			
				6 mos. postop.	120-130 240-300 145-160	1.017	19	Trace			
36 I. B.	F	43	12 yrs.	Pre-op.	260/150	1.022	45	0.6			
				1 mo. postop.	190/110					2b	
				1½ mos. postop.	240/160						
				6 mos. postop.	260/160	1.023	57	0.4			
37 F. R.	M	38	2 yrs.	Pre-op.	160-178 108	1.013	34	0.25	12		
				2 mos. postop.	170/110	1.014	27	0.2		2b	
				5 mos. postop.	170/110	1.015	27	0.22	16		Blood nonprotein nitrogen 56 mgm. per cent Blood nonprotein nitrogen 54 mgm. per cent
38 L. H.	M	46	3 yrs.	Pre-op.	195-215 116-130	1.028	96	Trace	1.1		
				3 wks. postop.	140/90	1.027	80		0	2b	
				6 mos. postop.	200/110		127	Trace			
39 W. B.	F	43	2 yrs.	Pre-op.	192-240 112-150	1.018	62				
				3 wks. postop.	200/110					2b	
				6 mos. postop.	236/140	1.022	71				
				9 mos. postop.	210-220 126-140	1.019	73				Slight edema present
40 C. V.	M	34	1 yr.	3 mos. pre-op.	230/150	1.019	47	0.33	0		
				1 wk. postop.	240/156	1.018	47	0.33	1.5	2c	
				3 wks. postop.	180/130						
				10 mos. postop.	220-250 150-170	1.011	16	0.3	0		
41 I. J.	F	55	5 yrs.	Pre-op.	268-300 130-170	1.019	38	Trace			
				3 wks. postop.	220-270					2c	
				10 mos. postop.	120 270 130-170	1.021	18	Trace			
42 E. H.	F	52	1 yr.	Pre-op.	230/130	1.024	69	Trace			
				3 wks. postop.	160/110		49	Trace		2c	
				15 mos. postop.	210-230 120-150						
43 C. W.	M	45	6 mos.	Pre-op.	210/120	1.027	91	Sl. tr.	0	2c	
				3 wks. postop.	140/90						
				10 mos. postop.	185-190 125-130	1.022	45	Trace	1.8		

TABLE 1—Continued

Case number and initials	Sex	Age at time of operation	Known duration of hypertension	Time of observation	Blood pressure	Concentrating ability of kidneys	Urea clearance	Urine		Classification in Table II	Remarks
								Proteinuria	Hematuria		
		years			mm. Hg	nonprotein specific gravity	per cent of mean normal	per cent	millions of r.b.c.'s per 12 hours		
44 F. S.	M	40	17 yrs.	Pre-op.	172-234	1.029	84	Trace	0	2c	
				3 wks. postop.	108-130 115-130 80-90		91	0	0		
				3 mos. postop.	194/124	1.025	82	0	0		
				7 mos. postop.	172/118	1.025	87	Trace	0		
45 H. Mc.	F	38	2 yrs.	Pre-op.	180/120	1.028	114	0.1	0	2c	
				1 wk. postop.	200/120						
				5 mos. postop.	205/130	1.021	89	Trace	0		
46 F. G.	M	35	3 mos.	Pre-op.	226-240	1.013	17	0.3	.9	2c	Nonprotein nitrogen 58 mgm. per cent
				4 wks. postop.	150-160		13				
				6 wks. postop.	160/120 250						
				8 wks. postop.	144-150						Died in uremia. Blood nonprotein nitrogen 114 mgm. per cent
47 L. B.	F	21	3 mos.	Pre-op.	220/146	1.017	40	1.0	2.4	2c	
				2 wks. postop.	166/122						
				6 wks. postop.	220/140	1.018	46	0.6			
				8 mos. postop.							Died in uremia
48 I. B.	F	45	3 yrs.	Pre-op.	155-182 110	1.037	95	Sl. tr.	0	3	
				6 mos. postop.	210/130	1.034	103	Sl. tr.	0		

imately normal; in others it rose to various levels even to the preoperative value. Following this secondary rise the blood pressure in some cases remained unchanged, in others it again decreased, sometimes rapidly sometimes over a period of months.

The effect of splanchnicectomy on the kidneys can best be considered in relation to the effect on the blood pressure. According to this relationship, the patients fall into different groups. Figures 1 to 8 illustrate the course of events in representative cases.

*Explanation of figures.* In each case the vertical line, topped by an arrow, represents the bilateral splanchnicectomy. The time charted to the left of this line refers to the known duration of hypertension. The postoperative events are charted at irregular intervals, properly designated. The blood pressure charted 2 or 3 weeks following splanchnicectomy represents the values at the time the patient was discharged from the hospital following operation. When the blood pressure fluctuated appreciably, the extremes are plotted and the intervening

space shaded. Although it is not a true representation, the values plotted for each occasion have been joined by a line in order to illustrate the trend of events.

In Figure 1 the changes in a patient (Case 1, W. L.) whose blood pressure after splanchnicectomy remained below 160/100 mm. Hg, are shown. The urea clearance was normal in this patient before operation, whereas the concentrating ability was greatly reduced. The lack of parallelism of these functions of the kidneys in this disease has been pointed out previously (6). This is an unusually wide variation, however. At the time of discharge from the hospital the urea clearance was below normal. A temporary decrease in urea clearance in the early postoperative period has been observed in a few other cases. Following splanchnicectomy, proteinuria promptly disappeared. Five months after operation the renal function had become entirely normal, and has remained so for more than two years.

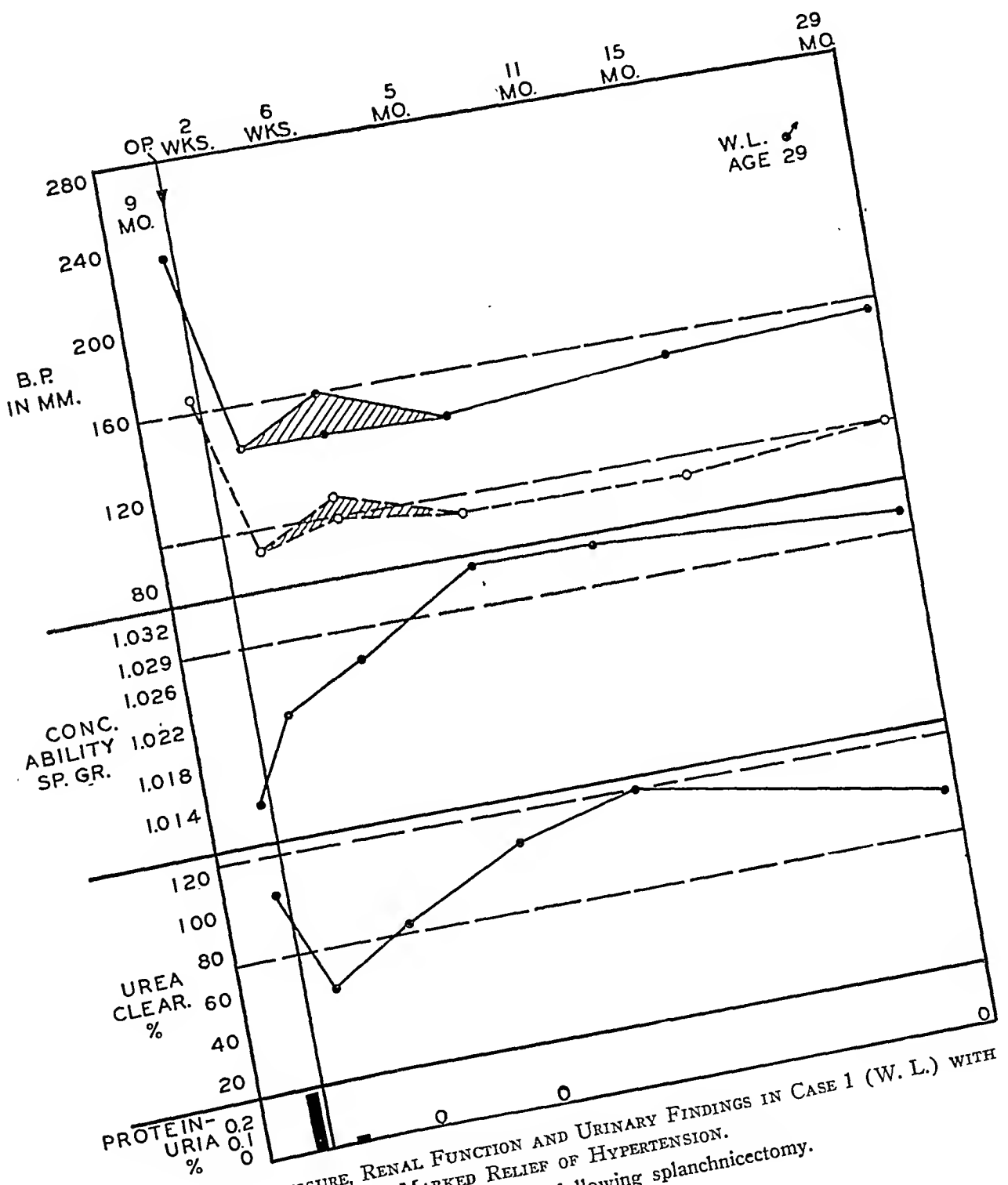


FIG. 1. BLOOD PRESSURE, RENAL FUNCTION AND URINARY FINDINGS IN CASE 1 (W. L.) WITH MARKED RELIEF OF HYPERTENSION. Concentrating ability returned to normal following splachnicectomy.

TABLE 11

The relation between renal function and blood pressure in 48 cases at the end of three months or longer after splanchnicectomy

	Num- ber	Per cent
1. Blood pressure decreased:	24	50
A. To 160/100 or less. (3 cases followed 6 to 29 months).....	6	12.5
a. Renal function normal before and after operation.....	1	
b. Renal function impaired before operation, returned to normal after operation.....	3	
c. Renal function remained slightly impaired (4 months) after operation.....	2	
d. Renal function decreased after operation.....	0	
B. Not to 160/100, but more than 60 mm. systolic, or more than 30 mm. diastolic.....	8	16.6
a. Renal function normal before and after operation.....	2	
b. Renal function impaired before operation, improved after...	6	
c. Renal function decreased after operation.....	0	
C. From 30 to 60 mm. systolic, or from 15 to 30 mm. diastolic.....	10	20.9
a. Renal function normal before and after operation.....	1	
b. Renal function impaired before operation, improved after...	5	
c. Renal function impaired before operation, unchanged after...	3	
d. Renal function decreased after operation.....	1	
2. Blood pressure essentially unchanged:	23	48
a. Renal function normal before and after operation.....	2	
b. Renal function impaired before operation, unchanged after.....	11	
c. Renal function impaired before operation, decreased after..... (to death in uremia, 2)	8	
d. Renal function impaired before operation, increased after.....	2	
3. Blood pressure increased:	1	2
Renal function normal before and after operation (6 months)		

In Case 2, M. P. (Figure 2) there was a prompt return to normal of both the urea clearance and concentrating ability, and the disappearance of proteinuria, with relief of the hypertension.

In Case 4, V. E. (Figure 3) there was no impairment of renal function before operation, and the efficiency of the kidneys remained normal, or promptly returned to normal after a temporary decrease following splanchnicectomy, when the hypertension was relieved. This case illustrates that if urinary abnormalities exist, they may disappear or greatly decrease.

In many patients, blood pressure was markedly reduced, but not below 160/100 mm. Hg (Group 1, B of classification above). In such cases, if renal function had been normal, it remained so;

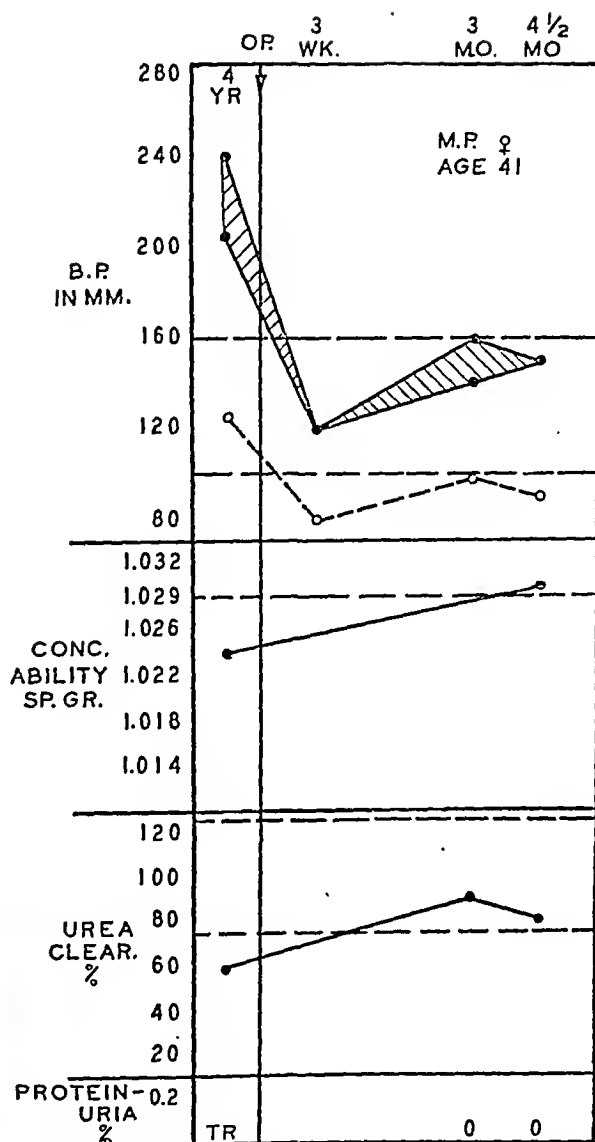


FIG. 2. DATA IN CASE 2 (M. P.), SHOWING A RETURN TO NORMAL OF THE CONCENTRATING ABILITY AND UREA CLEARANCE WITH RELIEF OF THE HYPERTENSION.

if it had been impaired, it improved and in some cases became entirely normal and the urinary abnormalities completely disappeared. Cases illustrating this effect are shown in Figures 4 and 5.

In some patients the blood pressure was decreased to a lesser degree (Group 1, C of classification above). In these persons, renal function usually improved if it had not been normal, or remained unchanged. In only one case (Case 24, L. B.) in this group was the concentrating ability found, fourteen months after operation, to be

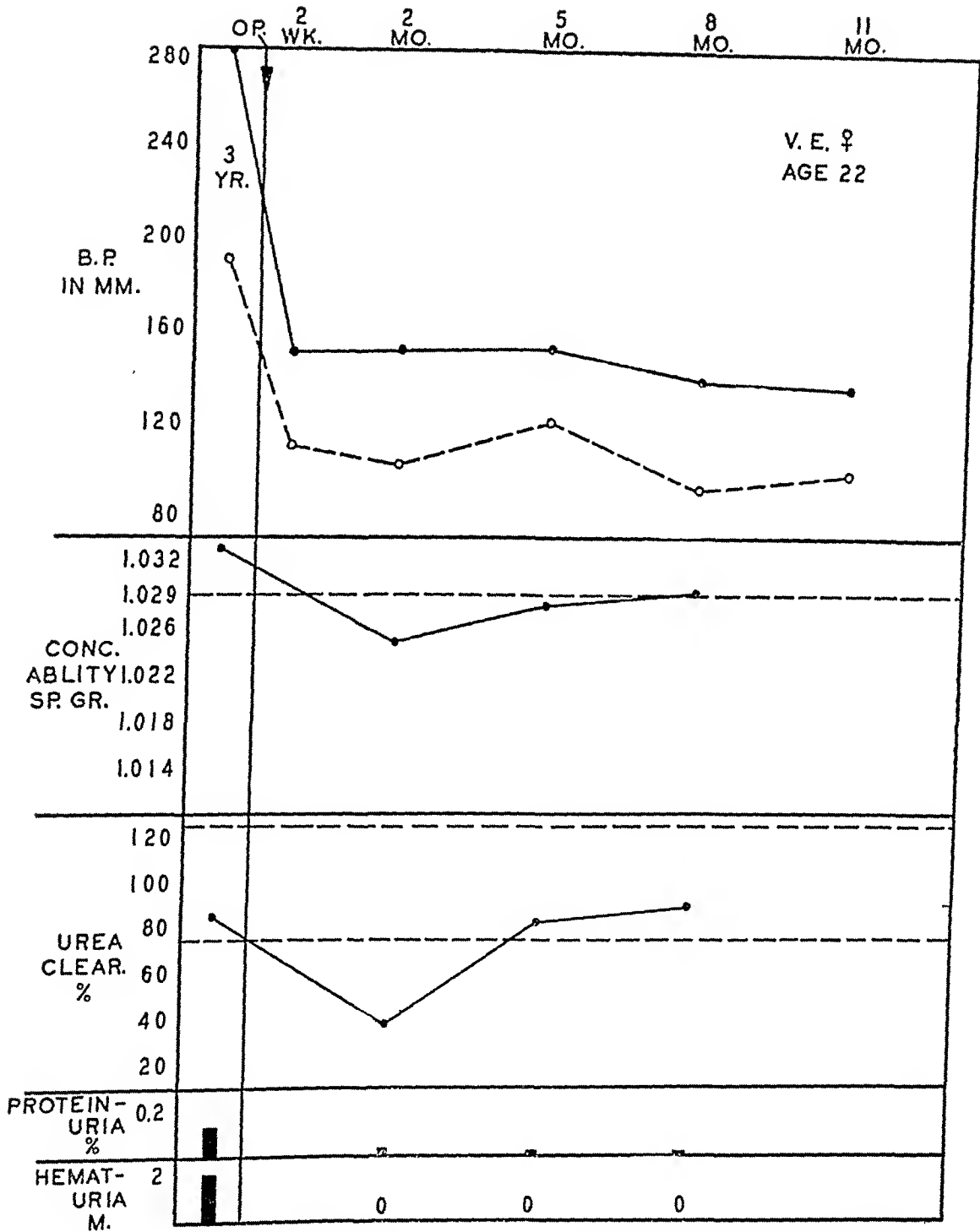


FIG. 3. DATA IN CASE 4 (V. E.), WHOSE RENAL FUNCTION WAS NORMAL BEFORE SPLANCHNICECTOMY.

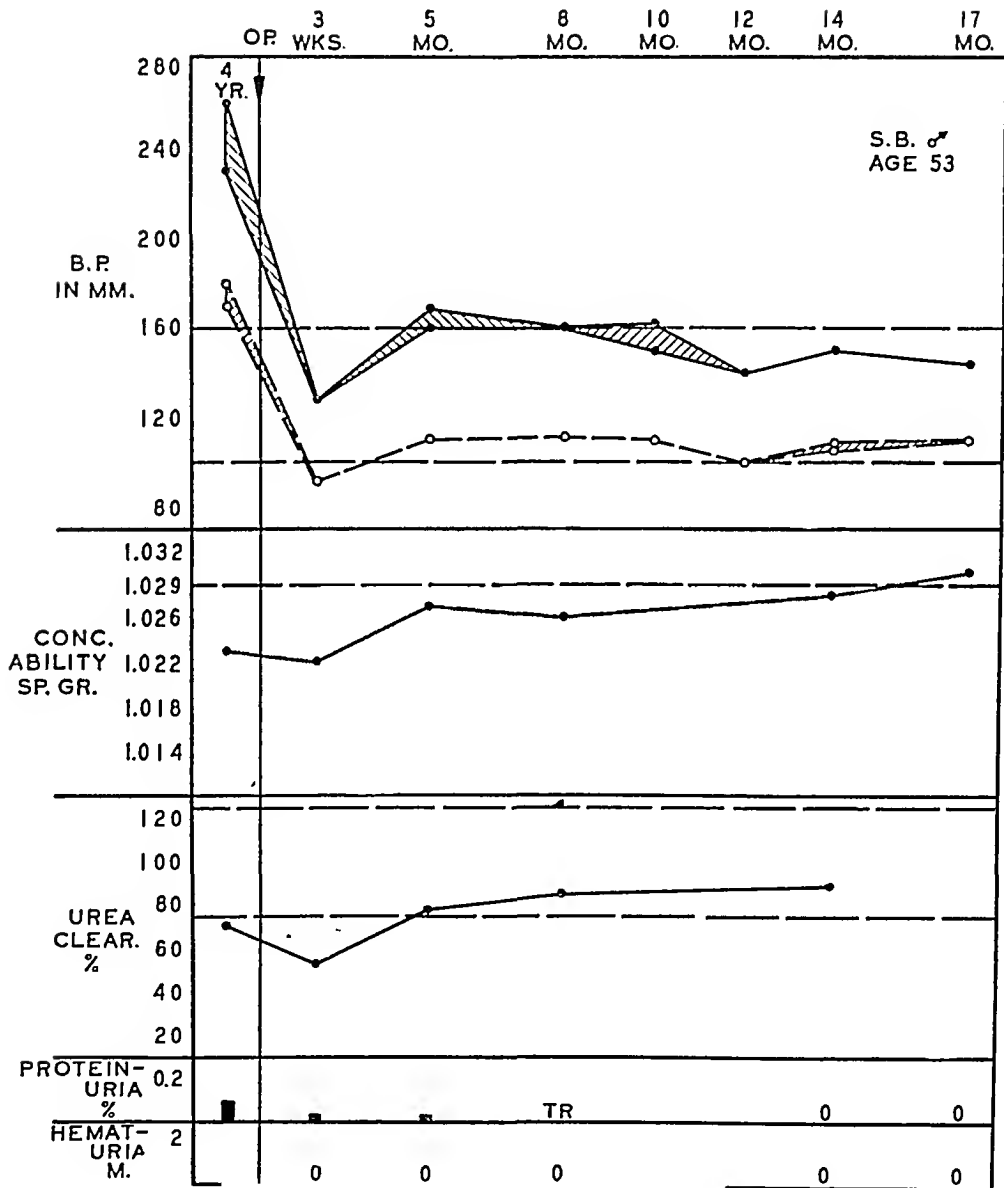


FIG. 4. CASE 8 (S. B.), SHOWING RETURN TO NORMAL RENAL FUNCTION AND NORMAL URINE WITH LOWERING OF BLOOD PRESSURE.

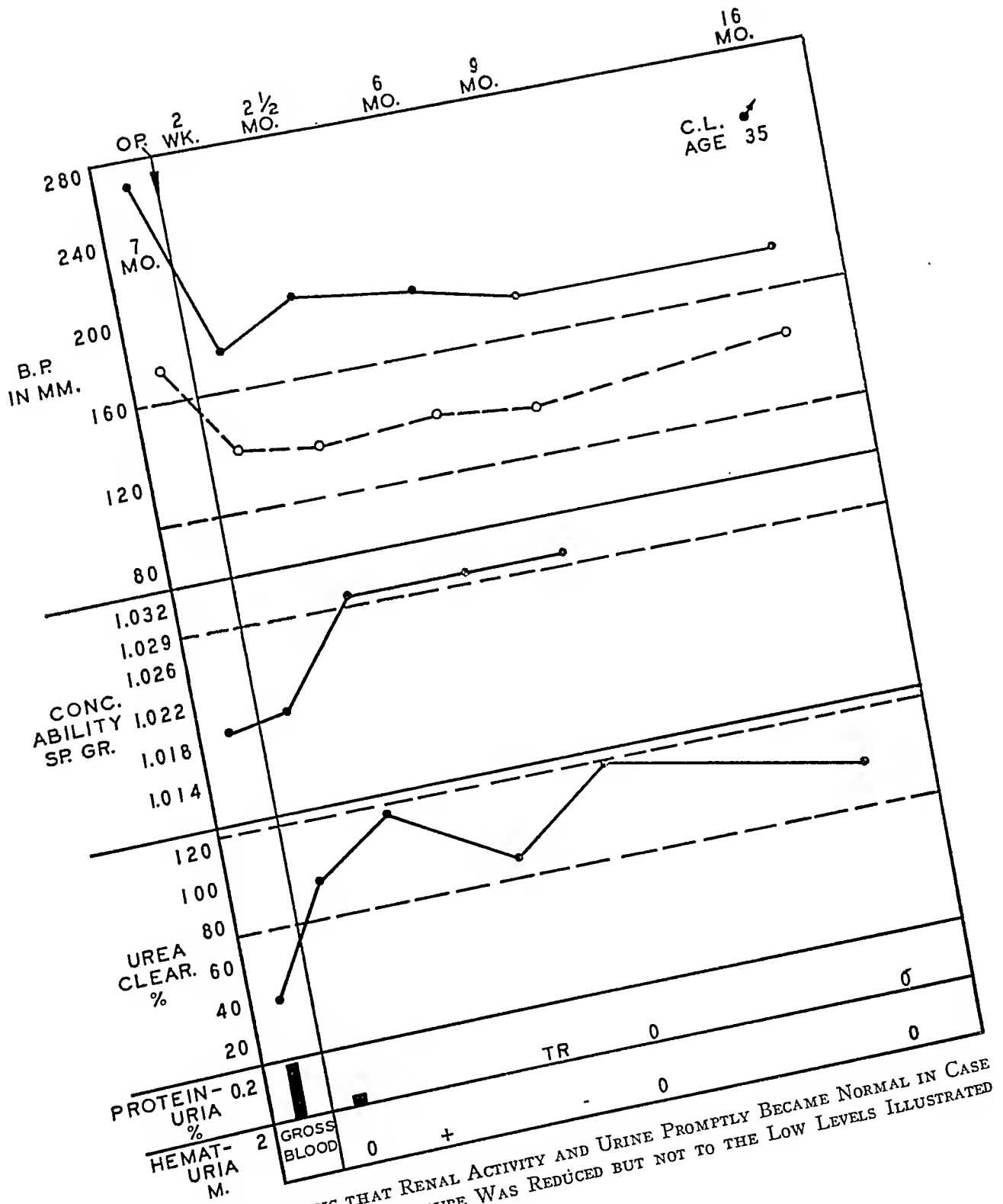


FIG. 5. SHOWING THAT RENAL ACTIVITY AND URINE PROMPTLY BECAME NORMAL IN CASE 7 (C. L.) WHOSE BLOOD PRESSURE WAS REDUCED BUT NOT TO THE LOW LEVELS ILLUSTRATED IN PREVIOUS CHARTS.

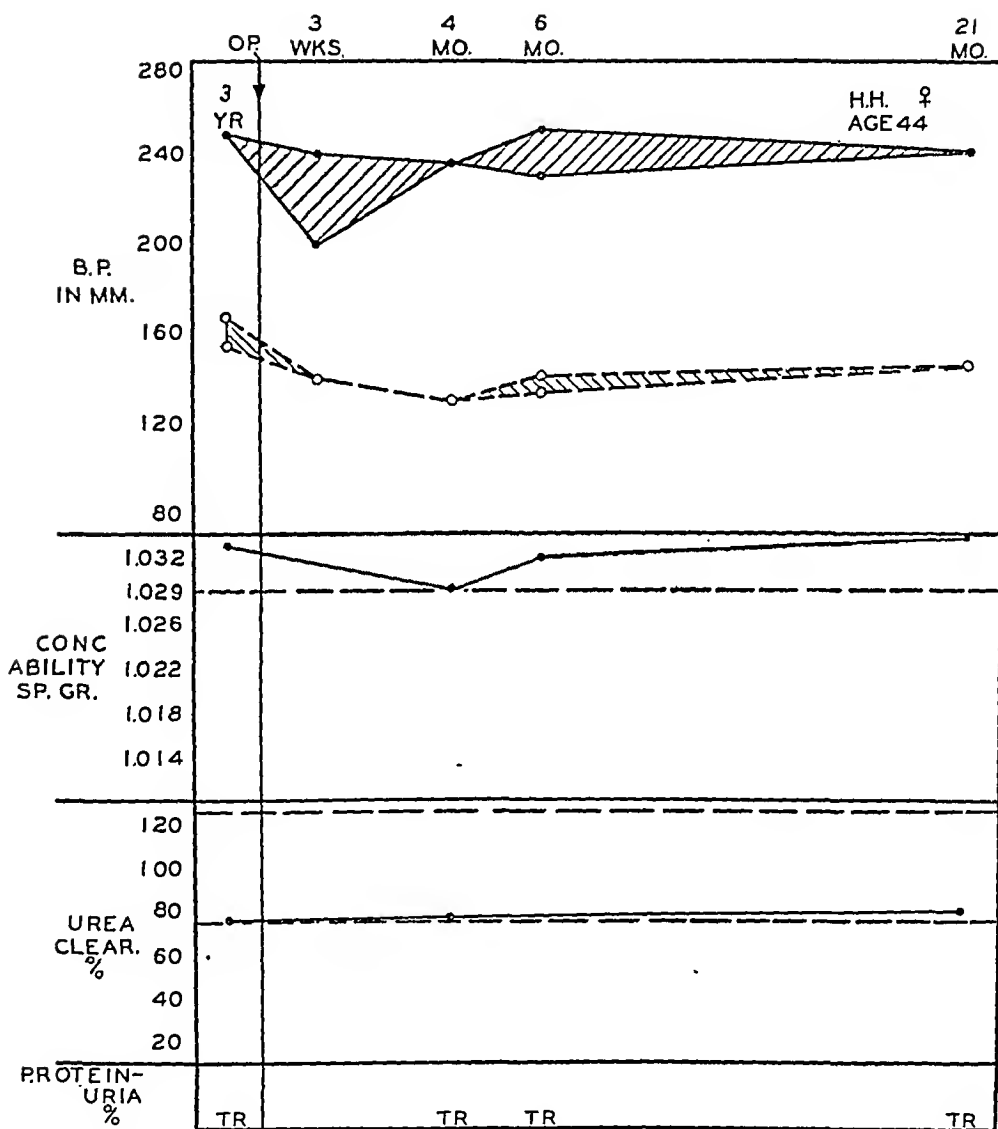


FIG. 6. SHOWING RENAL FUNCTION REMAINING NORMAL AFTER SPLANCHNICECTOMY IN CASE 25 (H. H.) WHO HAD NO LASTING DECREASE IN BLOOD PRESSURE.



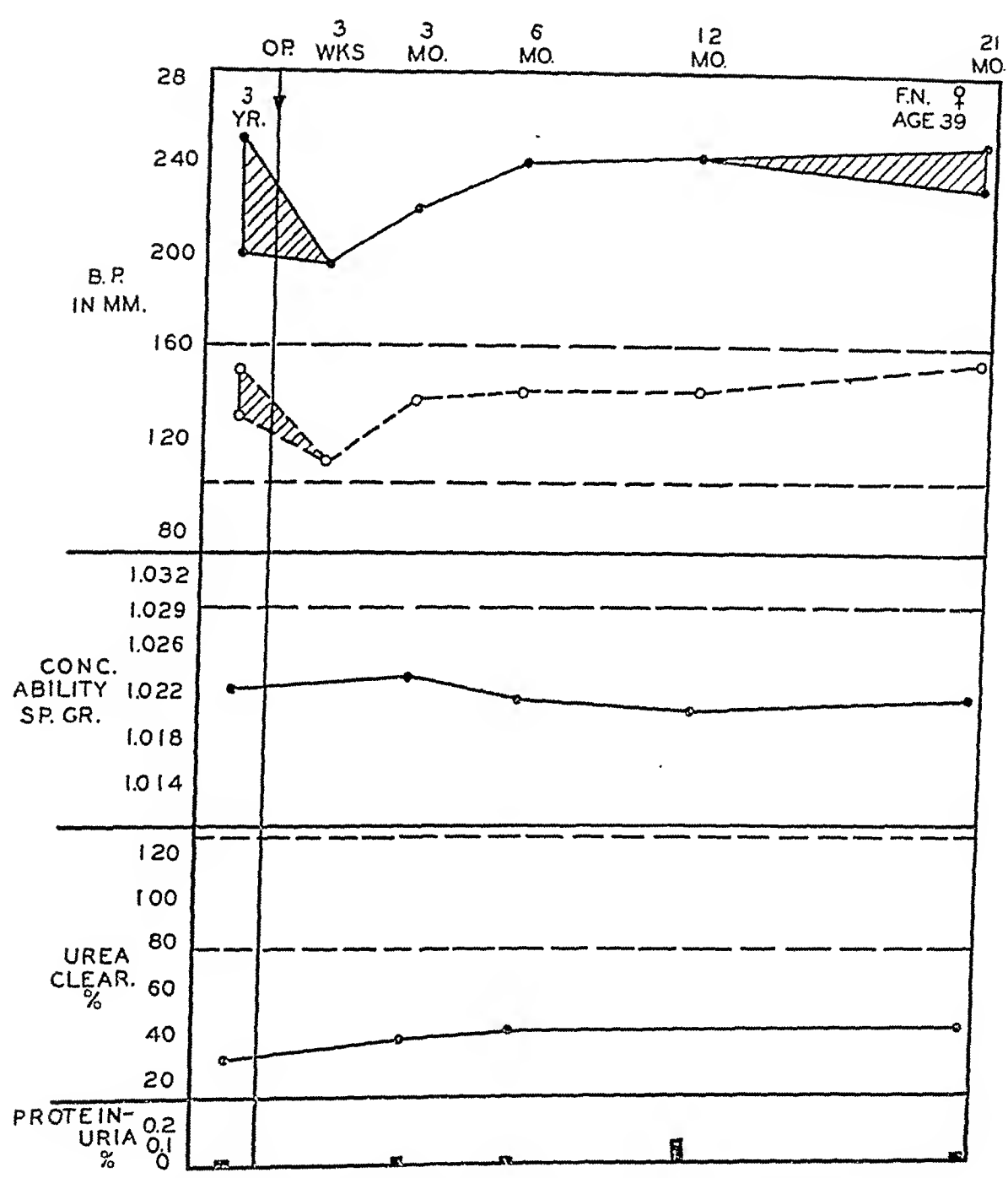


FIG. 7. REDUCED RENAL FUNCTION AND URINE REMAINED ESSENTIALLY UNCHANGED IN CASE 29 (F. N.) WHO HAD NO LASTING REDUCTION IN BLOOD PRESSURE.

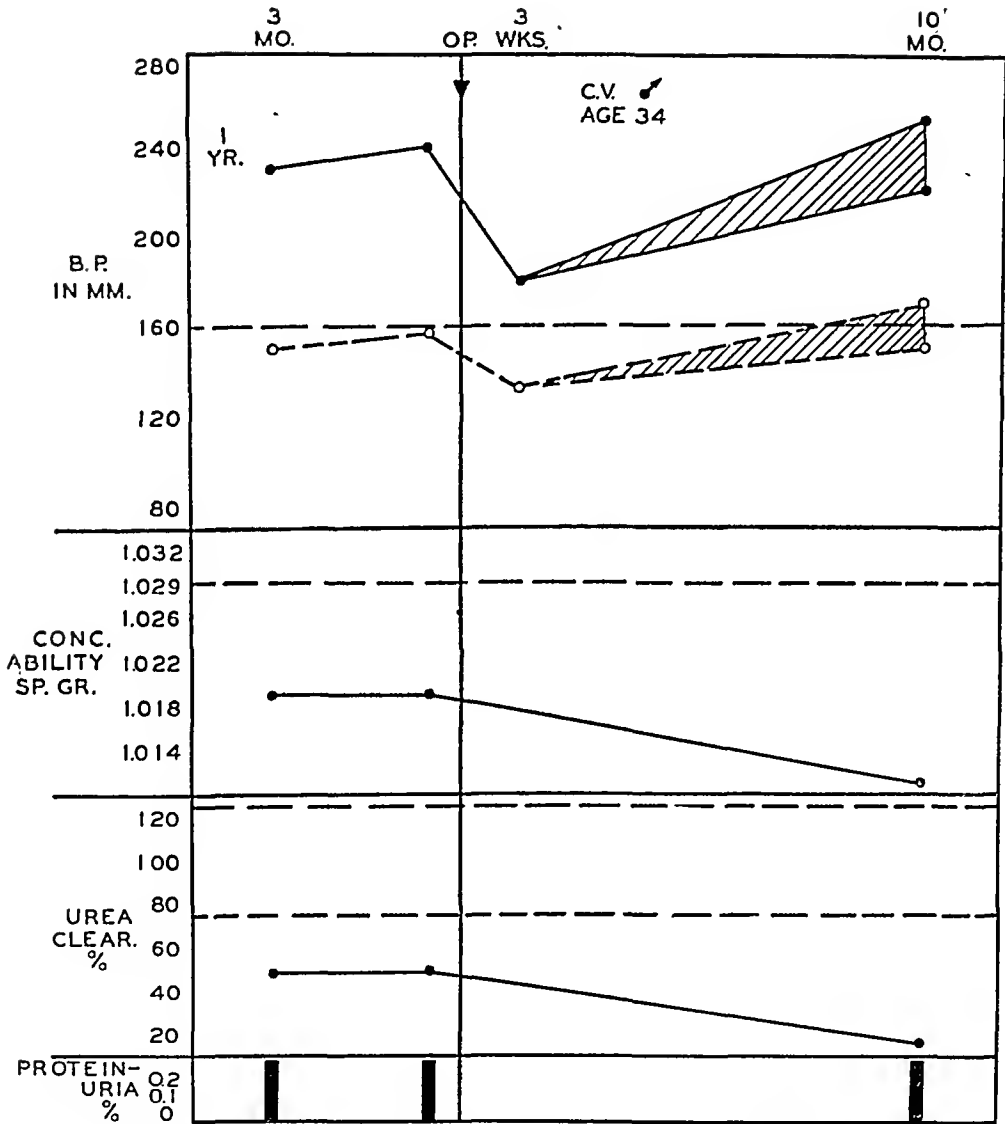


FIG. 8. SHOWING DECREASE IN RENAL FUNCTION AND PERSISTENCE OF PROTEINURIA IN CASE 40 (C. V.) WHOSE HYPERTENSION WAS NOT BENEFITED BY SPLANCHNICECTOMY.

slightly decreased. (The blood pressure at this time averaged 185/120 mm. Hg.)

In 50 per cent of the patients comprising this study, the blood pressure did not remain reduced following splanchnicectomy. In general, the renal function in this group of cases either remained unchanged, or became gradually worse, in a manner just as would be expected if splanchnicectomy had not been performed (Figures 6, 7 and 8). In two such cases (Case 46, F. G., and Case 47, L. B.) renal function decreased progressively until death occurred in uremia. Two persons in this group (Case 27, H. S., and Case 28, T. A.) showed an improvement in renal function.

#### DISCUSSION

We wish to emphasize that this study is not meant to convey statistics regarding the effect of splanchnicectomy on blood pressure or other clinical results. Many more patients have been operated upon than we have been able to study in the manner here described. Symptomatic changes, ocular fundus and cardiac changes, mortality, etc., must of course be considered in a complete appraisal of results of this form of treatment. We have included data on blood pressure herein, and have grouped the patients according to changes in their blood pressure, only for convenience in discussion of the effect on the kidneys. Those persons whom we considered to have small decreases in blood pressure, and especially those whom we have been able to follow only three to six months, may be found after a longer period of postoperative study to have no lasting reduction in blood pressure. It should be noted, however, that in four patients (Cases 4, 7, 8 and 1) the blood pressure has remained at a remarkably low level in comparison with preoperative values, for 11, 16, 17 and 29 months, respectively, following splanchnicectomy.

From the data presented, it is evident that splanchnicectomy performed on patients with primary hypertension and normal kidney function, *does not harm* the kidneys, or interfere with their functional efficiency as measured by concentration and urea clearance test, whether or not significant decrease in blood pressure results. Page and Heuer (7) have likewise found that denervation

of the kidneys which resulted from sectioning the anterior nerve roots from the 6th thoracic to the 2d lumbar segment in a patient with essential hypertension, in no way interfered with the renal function even though the blood pressure was reduced to normal. Page (8) has also shown that reduction in blood pressure induced by medication, or resulting from direct renal denervation of one kidney did not alter renal function as measured by urea clearance. All of these observations, therefore, show that in cases of primary hypertension, renal efficiency is not dependent on high blood pressure, as has been so commonly thought. Thus the "compensatory theory" of the cause of the elevated blood pressure in patients with primary hypertension is disproven.

We have repeatedly observed that when hypertension is greatly relieved by splanchnicectomy, renal function that has previously been impaired, improves, and may even return to normal. This improvement in kidney function has shown itself *both* by an increase in concentrating ability, and by an increase in urea clearance, in a number of cases.<sup>1</sup> This indicates to us that the impairment of renal function is caused by vascular constriction, and that if constriction is relieved by splanchnicectomy, renal activity is benefitted.

In some cases we have observed, as have Page and Heuer (9), an improvement in proteinuria and hematuria, out of all proportion to changes in renal function.

That the results obtained by splanchnicectomy should differ so widely in different patients, from complete relief of hypertension, in some cases, to complete failure in others, is striking. We are at present unable to predict or account for these differences. Contrary to the findings of Page and Heuer (9), however, we find a definite association between changes in renal function and in blood pressure in most patients. Certainly, renal disease with marked impairment of function may accompany hypertensive vascular disease, and when present, disappears following relief of the hypertension by splanchnicectomy.

<sup>1</sup> Page and Heuer (9) found an increase in concentrating ability in 2 of 5 patients *with nephritis* whose kidneys were denervated, but found no alteration in urea clearance.

# SUMMARY

Data regarding the renal status and blood pressure in 48 patients with hypertensive vascular disease treated by bilateral splanchnicectomy are presented. This surgical procedure has greatly relieved hypertension in some cases, has benefitted others to a lesser degree, and has not influenced the blood pressure in still others. In general, the changes in the kidneys were associated with changes in blood pressure. In those patients who had a significant and maintained decrease in blood pressure, urinary abnormalities decreased or disappeared, and the renal function, if it had been impaired, improved—in several cases it became entirely normal. In a few cases with less decrease in blood pressure, renal function remained unchanged following splanchnicectomy. When hypertension was lowered in patients having normal renal function, the efficiency of the kidneys remained normal. When hypertension was not favorably influenced, renal function remained unchanged, or gradually became worse as would be expected in unoperated cases.

These observations show that in cases of primary hypertension, satisfactory renal function is not dependent on the high blood pressure; that hypertension is not compensatory to measurable renal damage; that marked impairment of renal function may accompany hypertensive vascular

disease, and that striking improvement of renal function follows relief of hypertension brought about by splanchnicectomy.

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TABLE I

Cholesterol concentration, fluid intake and amount of drainage

Patient	Days after operation	Type of drainage	Cholesterol	Fluid intake	Drainage
			mgm. per cent	cc.	cc.
H. L.		Gallbladder	34	4760	100
	1			2060	30
	2			2240	50
	3		29	2200	50
	4			2300	150
	5		75	2550	75
	6			2320	100
	7		60	2750	100
	8			2230	75
	9		70	1920	50
	10			1840	75
	11		51	3250	
R. C.		Common duct	61	5210	
	2			5630	200
	3		57	4910	250
	4		96	2640	125
	6		86	2810	10
	7		103	2810	80
	8		67	2110	30
	9		93	1540	180
	10		61	2140	180
	11		83	1830	275
	12		86	1470	550
	13		83	1600	300
	14		90		

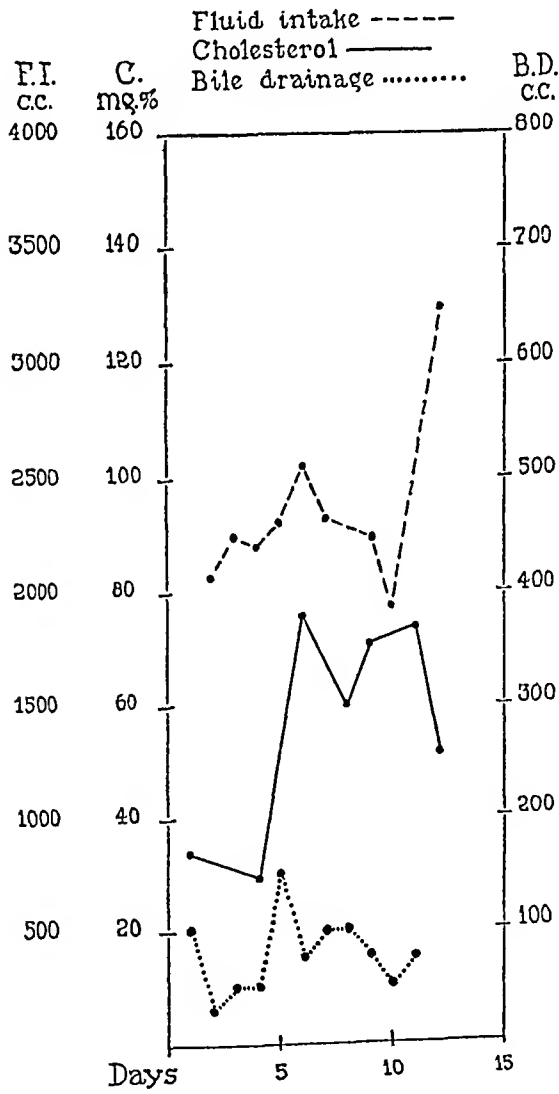
RESULTS

Daily analyses of cholesterol concentration in drainage bile show not only that there is considerable variation between different patients but also that in the same patient the concentration may vary widely from day to day. Attempts to correlate these variations with alterations in other factors such as fluid intake, amount of external bile drainage, etc., were unsuccessful. The only correlation which appeared significant was with the amount of liver damage demonstrable at the operation.

A. Variations in concentration in the same patient. In Table I and Figures 7 and 8 are given the concentrations of cholesterol and fluid intake and amount of external bile drainage in two pa-

obstruction of the lower end of that duct. Furthermore, for twenty-four to forty-eight hours after common duct intubation there may be some leakage of bile. In only two of the cases here reported was the common duct completely obstructed, and the bile drainage in these must closely approximate the complete daily twenty-four hour excretion. In all other instances only concentration of cholesterol could be determined. In a number of instances the first 2 or 3 specimens were contaminated with blood. Where this was the case the results were not considered in the following discussion.

In many instances the daily fluid intake of the patient was recorded, in order to determine its effect upon the concentration of cholesterol in the bile excreted. In some patients a portion of the collected bile was re-introduced through a Jutte tube, into the stomach. A record of this was kept and its effect on cholesterol concentration studied. Determinations of bile salt were made by the Gregory and Pascoe method (5). Cholesterol determinations were made as described by Riegel and Rose (6).



H.L.

FIG. 7. FLUID INTAKE, CHOLESTEROL CONCENTRATION AND BILE DRAINAGE IN PATIENT H. L.

TABLE II  
*Variations in cholesterol concentration with extent of liver damage*

Daily concentration of cholesterol (mgm. per cent)														
Slightly damaged				Moderately damaged				Badly damaged						
Patient E. P.	Patient C. W.	Patient F. B.	Patient B. G.	Patient B. D.	Patient M. F.	Patient D. C.	Patient F. M.	Patient R. G.	Patient H. I.	Patient W. R.	Patient N. A.	Patient B. M.	Patient R. S.	Patient W. W.
126	133	61	8	40	86	180	59	27	26	10	30	39	67	38
58	118	158	30	43	17	61	47	35	12	14	13	41	36	35
76	99	153	19	133	37	57	200		13	9	13	35	32	26
101	115	144	15	150	87	96	108		19	12	14	47	38	20
114	83	123	21	106	133	86	119		22	16	13	29	29	11
132	158	137	67	92	112	103	218		23	16	9	30	30	29
96	172	21	85	84	155	67			29	15	13	30	31	23
82	127	126	77	174	136	93			34	6	11	30	35	50
83	70	98	90	154	126	61			49	14	20	33	35	35
	200	62	133	108	146	83			53	9	11	39	23	
	225		71	90	159	86			53		25	47	50	
	240		47	133	154	83			47		9	62	39	
	211		154	170	90				54		5	72	36	
	139		57	152	169				48		14	48	19	
	152			123	110				70			80	14	
	214			76	123				65			63	27	
	82											49		
	128											47		
												45		
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												42		
												28		

tients studied. In the first patient, cholesterol concentration varied from 29 to 75 milligrams per cent, and in the second, from 57 to 103. One might expect that with greater fluid intake the volume of the collected bile would be greater, and the concentration of the bile constituents lower. No such relationship was found in these patients.

There is considerable daily variation in the amount of bile drainage through the collecting tube, but no relationship between this and the cholesterol concentration could be demonstrated.

The daily variations in cholesterol concentration agree with the data reported by Elman and Tausig (7) in the two patients they studied, and the data obtained by McMaster (8) from the dog.

There is a suggestion of correlation between the condition of the liver and its ability to function, and the concentration of cholesterol. In most of the patients the samples of bile collected in the first two or three days after operation, before the liver had begun to recover from the effects of obstruction, were lower in cholesterol

concentration than subsequent samples obtained at a time when hepatic function was improving.

*B. Variations in concentration in different patients.* When the patients were grouped according to extent of liver damage, as observed at operation, the seven patients known to have badly damaged livers had extremely low concentrations of cholesterol in the bile, while in the moderately or slightly damaged groups the concentrations on the whole were considerably higher. In Table II are given the data from the seven patients with a badly damaged liver, together with the data from four patients with a moderately damaged liver and four patients with a slightly damaged liver.

Table III shows that refeeding bile to the patients has little effect on cholesterol concentration, although Whipple (9) has shown that feeding bile salts to dogs increases cholesterol output.

One must take into account the fact that the bile which was refed, the patient's own bile, was not normal and almost certainly contained at least

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# THE NATURE OF CIRCULATORY COLLAPSE INDUCED BY SODIUM NITRITE<sup>1</sup>

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The study to be reported was undertaken with the purpose of throwing light on the mechanism of vasomotor collapse in man. During a previous investigation (1), it was noted that small doses of sodium nitrite, which in normal subjects would produce no symptoms and slight if any circulatory changes in the prone position, would in the upright position lead to progressive vasomotor collapse, often terminating in syncope. Return to the prone position was followed by immediate recovery without ill effects. By varying the dose of sodium nitrite and the angle of tilting the body, the duration as well as the degree of the circulatory collapse could be regulated. This method, then, offered an opportunity of recording observations on vasomotor collapse under well-controlled experimental conditions. This seemed particularly desirable in view of the fact that the study of vasomotor collapse, as it occurs in various diseases, is difficult because it develops unexpectedly in seriously ill patients and often terminates fatally.

## METHOD

Our primary aim was to observe simultaneously several aspects of the peripheral circulation before, during and after recovery from vasomotor collapse. The heart rate was counted by arterial palpation, by auscultation over the precordium, or from the pulse waves on the plethysmographic tracings. The arterial blood pressure was determined in the upper arm at heart level by the usual auscultatory method, using a mercury manometer. The arterial blood pressure and the pulse volume were registered by sphygmometric oscillometers.

The venous pressure was measured in the foot by the indirect method of Krogh, Turner and Landis (2). In some experiments the pressure in the femoral vein was determined by direct venous puncture, after the method of Moritz and von Tabora (3). All measurements are given in relation to the right auricle (second costal interspace). The skin temperature of the hands and feet was taken by means of a thermocouple.

The blood flow through the hands was measured by the plethysmographic method of Hewlett and Van Zwailenburg (4) as modified by Freeman (5). The average of 5 to 10 determinations was taken for each flow. The plethysmographs were arranged on adjustable stands which allowed free movement up or down as the subject was tilted. Usually the blood flow was determined through one hand at a temperature of 32° C., and through the other at 45° C. At 32° C. the physiological play of the vasomotor reflexes and other local vascular factors was maintained, whereas at 45° C. practically all the vasoconstrictor reflexes were eliminated, and as a result of complete vascular dilatation the maximal or potential blood flow was registered. The blood flow at 45° C. can thus be considered as an index of the cardiac output. In addition, we have determined by the method of Van Slyke and Neill (6) the femoral arterial and venous blood gases. The samples were taken and delivered under oil, and duplicate or triplicate determinations were made on each sample. The femoral arterio-venous oxygen difference under certain conditions is an index of the blood flow through the legs (7). Electrocardiograms were taken using the three standard leads and the fifth lead of Wolfarth and Wood (8). In some experiments the respiration was recorded by means of a Marey pneumograph.

When it was not feasible to obtain all the observations described above in a single experiment, the remaining observations were completed under identical conditions on another day.

Seven young adult subjects with normal cardiovascular systems were studied systematically. Isolated observations have been made in an additional larger group on certain aspects of collapse after nitrite. At least one hour after a meal the subject was placed on a tilting table in a horizontal position, and after all the apparatus had been adjusted he was allowed to rest from 45 to 60 minutes. The study of each subject consisted in the following procedures carried out on different days. After first obtaining resting values in the horizontal position, observations were made upon (1) the effects of elevation to an upright position (75°) for 45 minutes or longer, followed by a return to the prone position; (2) the effect of oral administration of 0.12 to 0.18 grams (2 or 3 grains) of sodium nitrite in the horizontal position for one hour; (3) the effect of the same amount of sodium nitrite followed in 10 to 20 minutes by elevation to the upright position. While in the upright position the subjects were urged to remain motionless. At the height of the vasomotor collapse, which was usually

<sup>1</sup> This investigation was aided in part by a grant from the Josiah Macy, Jr., Foundation.



associated with syncope, the subject was promptly brought back to the horizontal position and the observations were usually continued up to an hour.

### RESULTS

The symptoms and clinical signs exhibited, as well as the changes observed in the cardiovascular system, were remarkably uniform in all subjects. The only essential variation in different persons consisted in the amount of nitrite and the subsequent duration of standing required to produce complete collapse of the circulation. The susceptibility to vasomotor collapse did not necessarily depend on the physical state. Some of the robust and physically trained subjects developed vasomotor collapse and syncope promptly after relatively small amounts of nitrite.

The series of observations made on D. M., a tall, robust subject of 28 years, serve to illustrate the results obtained on all subjects. Figure 1

presents the response to the upright position without nitrite. The maximal blood flow (at 45° C.) through the hand remained essentially unaltered. The arterial pulse pressure became somewhat smaller as a result of the slight fall in the systolic and the rise in the diastolic pressures, which is the physiological response to standing. The venous pressure in the foot became elevated and later showed a moderate though progressive fall, but remained above the hydrostatic level of the heart. The heart rate increased. The subject remained symptom free. After the return to the horizontal position the circulatory measurements resumed their previous values.

Figure 2 presents the responses of the same subject, while remaining in the horizontal position, to 0.18 gram (3 grains) of sodium nitrite. There was slight, if any, fall in the potential blood flow to the hand, no essential change in the arterial

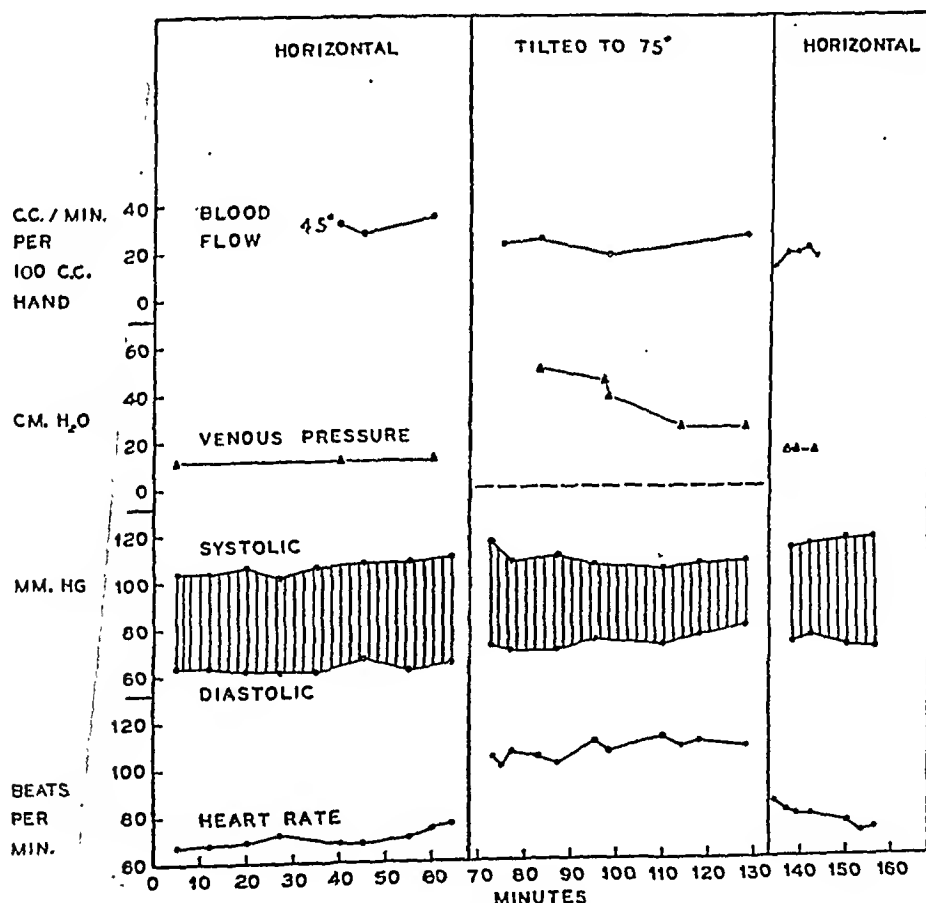


FIG. 1. SUBJECT D. M. EFFECT OF TILTING TO 75° ON THE BLOOD FLOW THROUGH THE HAND AT 45° C., ON THE VENOUS PRESSURE IN THE FOOT, THE ARTERIAL BLOOD PRESSURE AND HEART RATE.

All measurements of venous pressure are given in relation to the level of the right auricle (second costal interspace).

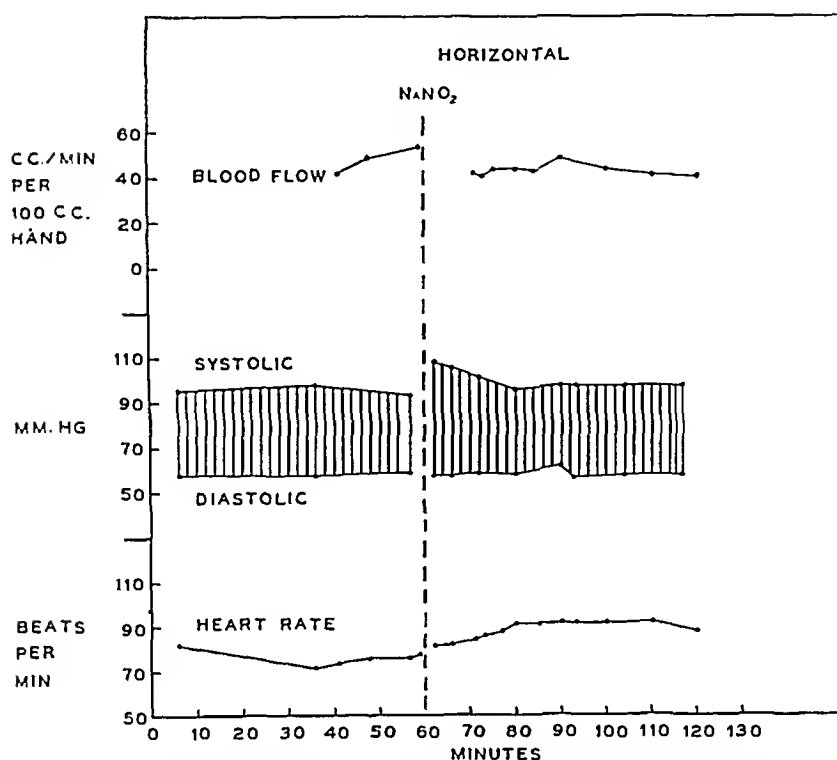


FIG. 2. SUBJECT D. M. EFFECT OF 0.18 GRAM OF SODIUM NITRITE ON THE BLOOD FLOW THROUGH THE HAND AT 45° C., ON THE ARTERIAL BLOOD PRESSURE AND THE HEART RATE.

pressure, and only a very slight rise in the heart rate. There were no subjective symptoms.

Figure 3 demonstrates his responses after the same oral dose of nitrite and subsequent elevation to the upright position. Ten minutes after the ingestion of nitrite and while still in the horizontal position, there was again only a slight fall in the blood flow and moderate rise in the cardiac rate. Following elevation, however, marked changes occurred in rapid succession. For about 5 minutes the subject remained symptom free. Thereafter he yawned occasionally, the intervals between yawns becoming progressively shorter toward the end of the standing period. The respirations became deeper and at times assumed the character of sighing. He became restless. Belching and increase in peristaltic sounds appeared. First warm and later cold perspiration broke out over the face and extremities, and finally it became beaded and profuse over the entire surface of the body. The skin became first slightly cyanotic and in about 20 minutes it was ashen grey.

The subject appeared drowsy. The pupils were dilated. At this point the picture corresponded in every respect to clinical vasomotor collapse.

The moderately lowered blood flow, which developed right after the tilting, was well maintained until shortly before complete collapse and syncope, when it rapidly fell to zero. The blood flow became more and more influenced by the deep respiration, as well as by the sighing and yawning. The arterial pulse pressure became quite narrow soon after tilting, mainly as a result of a fall in the systolic pressure. The diastolic pressure was well sustained. The pulse at the wrist became small and thready, while the carotid pulsation was still good. Finally, the radial pulse became imperceptible. It was of interest to note that after each yawn or sigh the pulse was instantly restored, to disappear again after several beats. The occurrence of yawning or sighing could often be foretold from the preceding disappearance of the pulse. The venous pressure throughout the standing fell rapidly until it

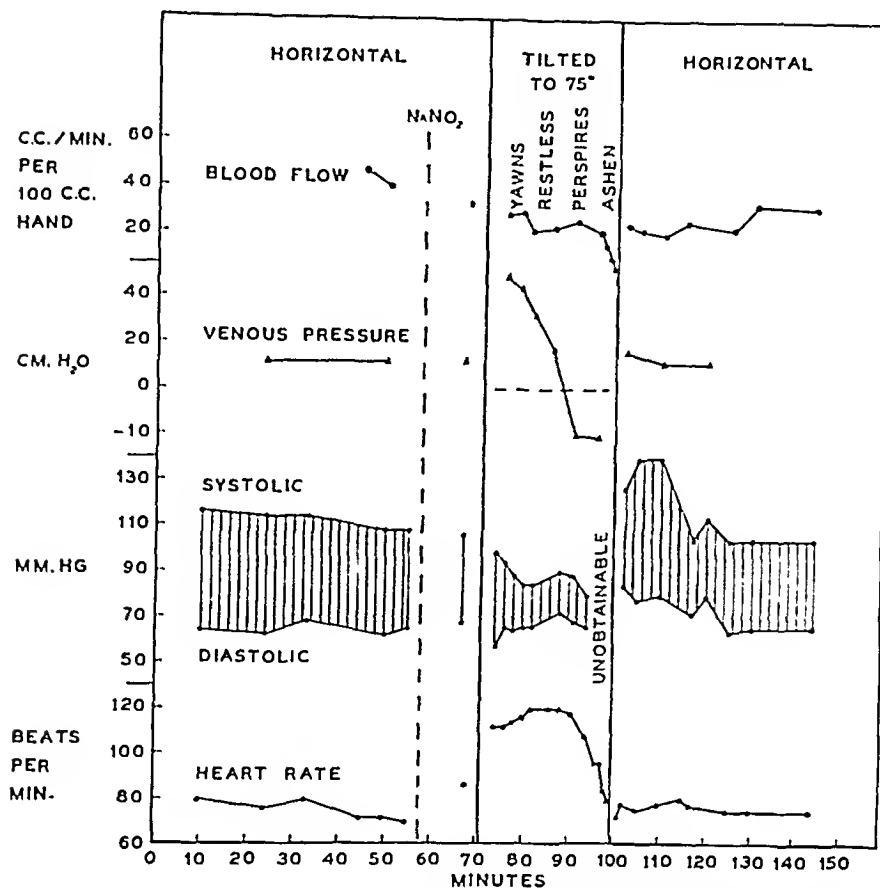


FIG. 3. SUBJECT D. M. EFFECT OF ADMINISTRATION OF 0.18 GRAM OF SODIUM NITRITE, FOLLOWED BY TILTING TO 75°, ON THE BLOOD FLOW THROUGH THE HAND AT 45° C., ON THE VENOUS PRESSURE IN THE FOOT, THE ARTERIAL BLOOD PRESSURE AND THE HEART RATE.

After 29 minutes at 75° the subject fainted. All measurements of venous pressure are given in relation to the right auricle.

reached a level which was below the hydrostatic level of the right auricle. This indicates that the column of blood in the inferior vena cava stood at a level below the right auricle. The rate of the heart became rapid and remained at about 120 per minute until shortly *before* the syncope, when it slowed markedly to about 80. At this time the blood pressure was not obtainable. Finally, the vision became dim and manifestations of loss of muscular power and of unconsciousness appeared, whereupon the patient was immediately returned to the horizontal position. Within 15 to 20 seconds he regained consciousness and all symptoms subsided.

Coincident with the rapid subjective improvement after a return to the horizontal position, all aspects of the circulation also promptly returned to normal. The systolic and diastolic blood pressure, in spite of the normal heart rate, actually became temporarily elevated.

The plethysmographic blood flow and respiratory tracings of P. C., a 29-year-old subject, are presented on Figure 4. The symptoms and signs of this subject, who received 0.18 gram (3 grains) of sodium nitrite and was then tilted upright, were essentially the same as those described above. In the prone position the blood pressure and the maximal blood flow (at 45° C.) through the hand showed no change after the administration of nitrite. After elevation to the upright position the pulse pressure became small and the pulse rapid and thready. The venous pressure fell and reached a negative level before syncope. Two minutes before the fainting the blood flow was still about 60 per cent of the original level, in spite of a pulse pressure of only 16 mm. Hg and a cardiac rate of 154 per minute. Thirty seconds before fainting, however, no blood flow or arterial pressure was obtainable. The respirations became deep and rapid and the cardiac rate slowed

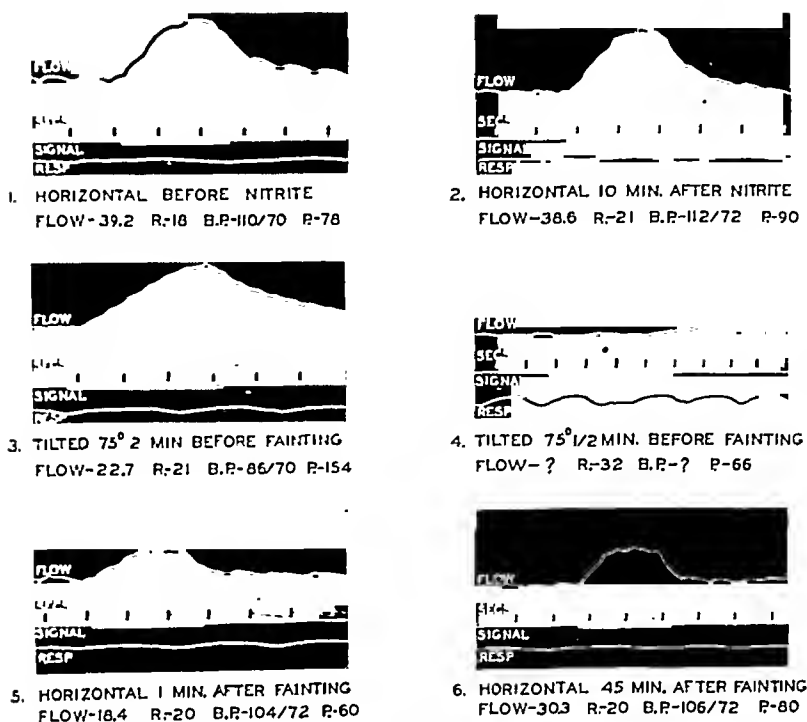


FIG. 4. SUBJECT P. C. PLETHYSMOGRAPHIC RECORDS OF BLOOD FLOW IN THE HAND AT 45° C. AND RESPIRATORY TRACINGS AFTER 0.18 GRAM OF SODIUM NITRITE, FOLLOWED BY TILTING TO 75°.

After 16 minutes at 75° the subject fainted.

abruptly to 66. Within 25 minutes after resuming the horizontal position the blood flow returned to normal.

Because of the slowing of the heart rate just before syncope, the question arose as to whether the late and sudden fall in blood flow, and hence the final syncope, depended on this cardiac slowing. The experiment outlined on Figure 3 was therefore repeated with the intramuscular administration of 2 mgm. of atropine sulphate. This eliminated the cardiac slowing without, however, altering the manifestations of vasomotor collapse. In the light of this finding, it is our contention that cardiac slowing is a secondary manifestation and is dependent on stimulation of the vagus centers by cerebral anoxemia. The degree of vagal manifestations varied in different subjects, and in some was not present even though fainting occurred.

The observations made on S. M., a 19-year-old subject, are typical of those experiments in which the blood flow was measured simultaneously in

both hands. Figure 5 represents the response to tilting without nitrite. The blood flow in the hand at 32° C., as expected, was slower than in the warm hand (45° C.) and showed a somewhat greater decrease with tilting, presumably due to vasoconstriction. Ultimately, however, the flow became about the same in both hands. As indicated in Figure 6, in the dilated hand the maximal flow decreased after nitrite, while it remained the same in the cooler hand with normal vasomotor regulation. Figure 7 shows the responses after 0.12 gram (2 grains) of nitrite, followed by tilting. Here the blood flow in the dilated hand showed a considerable decrease immediately after tilting, while in the hand with normal vasomotor regulation it became unusually slow, as a result of vasoconstriction. On returning the subject to the horizontal position, the rise in the cool hand was particularly rapid, reaching the level of the maximal flow, indicating an active vasodilatation in the cool hand. These responses exemplify the observations made in this and in other studies

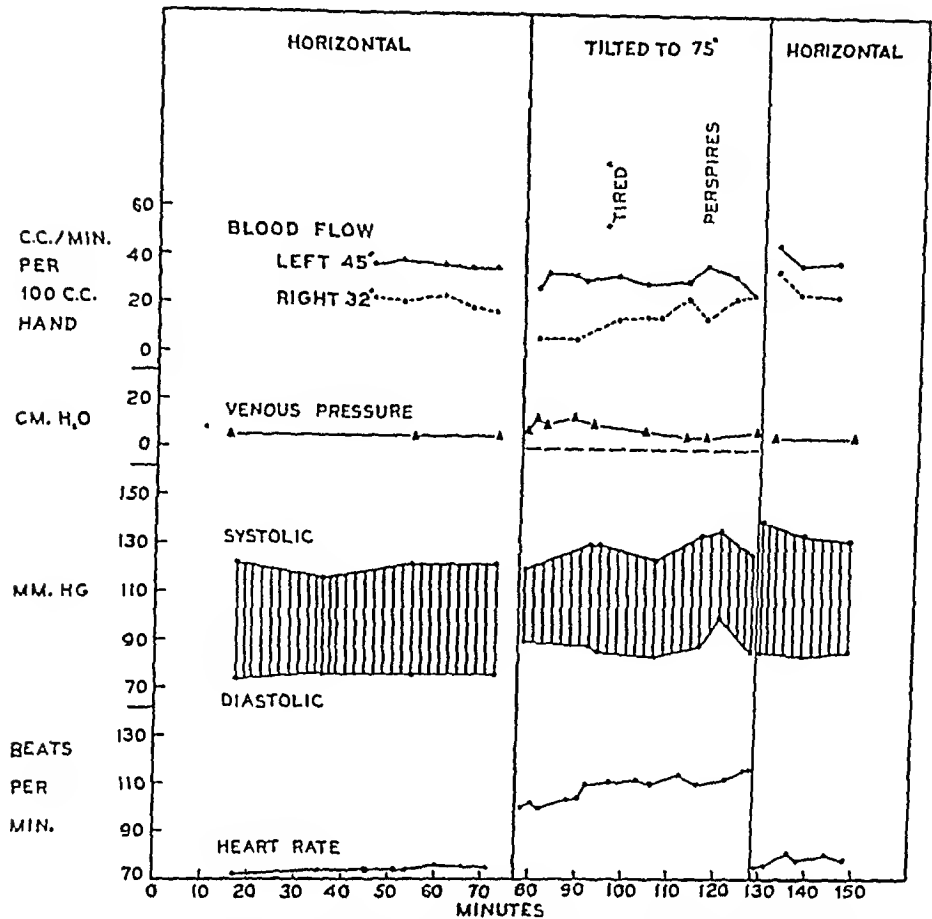


FIG. 5. SUBJECT S. M. EFFECT OF TILTING TO 75° ON THE BLOOD FLOW THROUGH THE HANDS, ONE AT 45° C. AND THE OTHER AT 32° C., ON THE VENOUS PRESSURE IN THE FOOT, THE ARTERIAL BLOOD PRESSURE AND THE HEART RATE.

All measurements of venous pressure are given in relation to the right auricle.

that the cool hand with normal vasomotor regulation shows greater spontaneous variations in blood flow than the dilated hand. Spontaneous vasoconstrictor responses were frequently noted in the cool hand during tilting. The rest of the circulatory changes, as well as the other manifestations of vasomotor collapse, were similar to those described in the first subject. Although, in Subject S. M., syncope developed as soon as 12 minutes after the tilting, in some subjects the manifestations of collapse without syncope have been maintained for as long as an hour or more by varying the dose of nitrite and the degree of tilting.

In order to ascertain the effect of nitrite and tilting on the *blood flow in the legs*, the femoral arteriovenous oxygen difference was determined at different times under the experimental conditions described above. Table I presents the re-

TABLE I  
*Effect of 0.18 gram of sodium nitrite, followed by tilting to 75°, on the arteriovenous oxygen difference of the femoral blood of Subject P. C.*

	Volumes per cent
Horizontal—control.....	5.79
At 75°, 2 minutes after tilting, 14 minutes after NaNO <sub>2</sub> .....	7.59
At 75°, 19 minutes after tilting, 31 minutes after NaNO <sub>2</sub> (fainting).....	12.26
Horizontal, 15 minutes after tilting back to horizontal.....	7.58

sults of a typical experiment. As indicated, the oxygen difference, and hence the blood flow, decreased slightly soon after the tilting and reached a level of 12.26 volumes per cent at the time of the fainting. The degree of slowing, however, is not unusual, because similar values have been obtained in control observations after standing only (7).

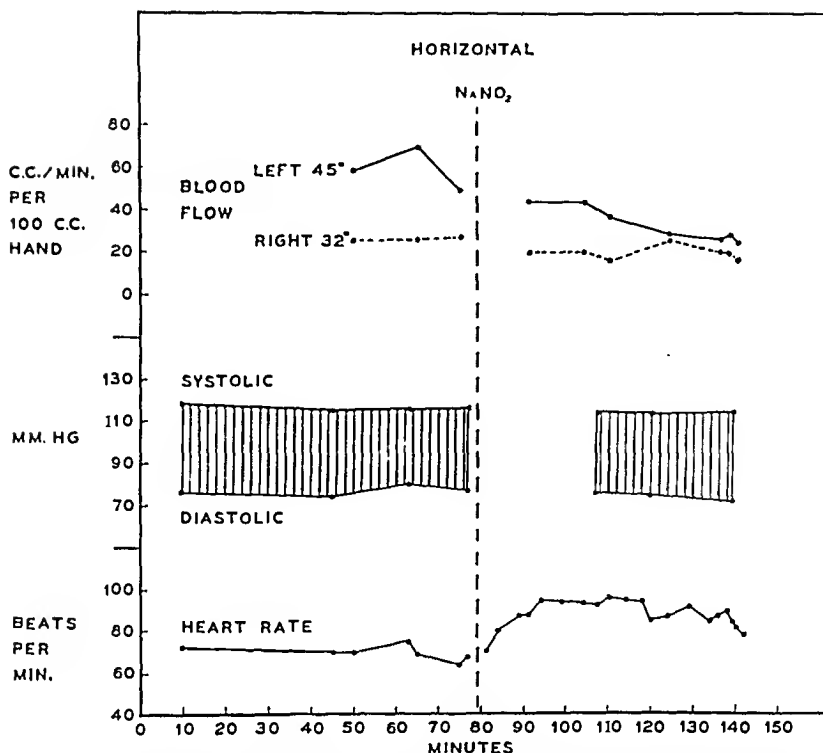


FIG. 6. SUBJECT S. M. EFFECT OF 0.12 GRAM OF SODIUM NITRITE ON THE BLOOD FLOW THROUGH THE HANDS, ONE AT 45° C. AND THE OTHER AT 32° C., ON THE ARTERIAL BLOOD PRESSURE AND THE HEART RATE.

The *pulse volumes*, as measured with oscillo-meters, revealed a perceptible increase after the administration of nitrite, with the subject in the prone position, an observation which is in accord with the known effect of nitrite on the larger arteries (1). After tilting, with or without nitrite, there was a decrease in the arterial pulse volumes of both the arm and the leg. This decrease paralleled the pulse pressure and the character of the pulse by palpation.

The changes in the *skin temperature* were variable, even though care was taken to maintain constant room temperature, and repeated observations were made. We were unable to correlate these changes with any of the other measurements of the circulation. In some of the experiments, a fall in skin temperature was found on standing, but this was not uniformly true.

In view of the small brachial pulse pressure, rapid heart rate and markedly decreased flow through the hand toward the end of the vaso-motor collapse, it became of interest to ascertain

whether the *electrocardiogram* would reveal any changes indicating anoxemia of the heart. It was rather unexpected to find that the changes, even during the height of the collapse, were but slight and mainly in the fifth lead, consisting of considerably increased amplitude of the T wave, slightly out of proportion to the increased rate, as indicated in Figure 8. This absence of change in the electrocardiographic complexes is in harmony with lack of symptoms referable to cardiac anoxemia.

#### DISCUSSION

In this study, a correlation has been made between a group of clinical symptoms and signs and the technical measurements of the cardiovascular system and the circulation. The syndrome presented by the subjects was identical with that exhibited by patients with a pronounced degree of vasomotor collapse, such as is apt to occur in pneumonia and other infectious diseases, in anaphylactic shock, abdominal perforation and in

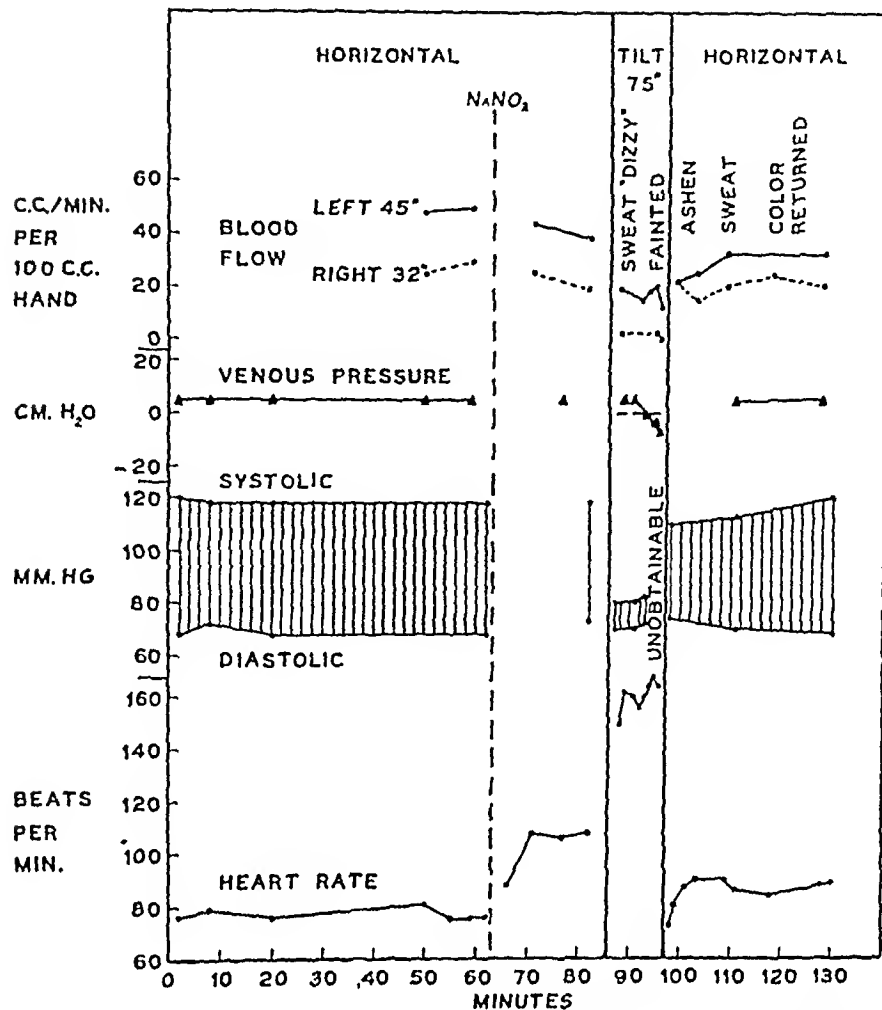


FIG. 7. SUBJECT S. M. EFFECT OF 0.12 GRAM OF SODIUM NITRITE FOLLOWED BY TILTING TO 75° ON THE BLOOD FLOW THROUGH THE HANDS, ONE AT 45° C. AND THE OTHER AT 32° C., ON THE VENOUS PRESSURE IN THE FOOT, THE ARTERIAL BLOOD PRESSURE AND THE HEART RATE.

All measurements of venous pressure are given in relation to the right auricle.

other conditions. The significance of the study lies in the fact that continuous observations have been made on several aspects of the circulation in subjects with normal cardiovascular and nervous systems, during the induction of collapse by an agent which exerts no primary effect on the heart. Peripheral failure of the circulation was studied, therefore, in its pure form under controlled conditions.

The primary changes in the circulation, which always preceded the symptomatic manifestations of collapse, consisted in tachycardia, small arterial pulse pressure with small pulse volume (thready pulse), fall in the venous pressure and arteriolar vasoconstriction, as indicated by moderately decreased blood flow of the cool, but not of the

warm, hand. The small pulse pressure was caused mainly by a fall in the systolic pressure, with fairly well maintained or even elevated diastolic pressure. Fall in the diastolic pressure was observed during the advanced state of collapse. It is of particular significance that in the presence of these circulatory changes the maximal blood flow was but moderately decreased, and the subject remained free of symptoms. As the above described circulatory changes continued, however, symptoms of collapse appeared which were associated with a progressive decrease in the maximal blood flow. When it had decreased to from 20 to 40 per cent of its normal value, pronounced manifestations of circulatory collapse developed, although consciousness and other vital bodily re-

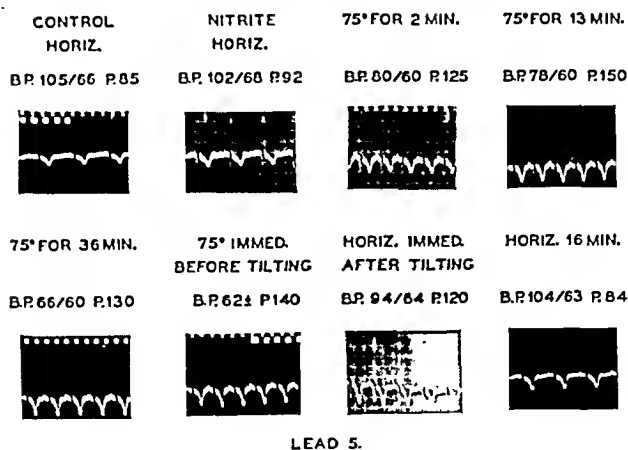


FIG. 8. SUBJECT D. M. ELECTROCARDIOGRAPHIC RECORDS AFTER THE ADMINISTRATION OF 0.18 GRAM OF SODIUM NITRITE, FOLLOWED BY TILTING TO 75°.

Subject remained at 75° for 52 minutes, at the end of which he felt weak and dizzy.

sponses were still maintained. Further diminution of the blood flow finally led to fainting, usually associated with rapid fall of the diastolic as well as of the systolic pressure. This syncope exhibited all the characteristics of the vasovagal type (9, 10).

The collapse studied furnishes an example of a bodily state in which nervous manifestations arise secondarily to changes in the vascular system. The marked elevation of the cardiac rate, the change in arterial pressure and the vasoconstrictor response observed in the hand occurred practically instantaneously after the tilting and before the appearance of subjective symptoms. The elevation of the cardiac rate, which in some cases attained a level of 140 to 160 per minute, and the vasoconstrictor response are attributed to the stimulating effect of the lowered systolic pressure on the carotid sinus and aortic depressor reflex mechanisms. Lowering of the arterial pressure in these two vascular areas induces a nervous response leading to tachycardia and vasoconstriction, as has been shown by Hering (11), Heymans (12) and others. The changes observed subsequently are attributed to a progressively increasing ischemia of the medullary centers. It is of particular interest to note that at this time hyperactivity of both sympathetic and parasympathetic functions occurred *simultaneously*, although

with the approach of maximal medullary ischemia the parasympathetic manifestations became predominant. Thus tachycardia, vasoconstriction and dilatation of the pupils were observed simultaneously with sweating, yawning, belching, nausea, cramps (indicating pyloric spasm) and increased peristalsis. Just before the onset of syncope, bradycardia often appeared. This bradycardia and some of the other evidences of parasympathetic overactivity are considered as secondary phenomena to the collapse and syncope, since the latter syndromes occurred in some subjects without these parasympathetic manifestations. Furthermore, atropine abolishes the bradycardia without essentially influencing the course of collapse and syncope.

From our data and from what we know of experiments on animals it is impossible to state whether this simultaneous overactivity of both the sympathetic and parasympathetic systems depends entirely upon increasing medullary ischemia stimulating both types of centers directly, or upon a combination of this effect and the action of the aortic and carotid sinus depressor reflexes. In response to the drop in systolic pressure, the latter reflexes induce overactivity of certain sympathetic functions, and at the same time inhibition of parasympathetic functions. The simultaneous advanced ischemia of the medulla acts as a powerful



stimulus to the vagal (parasympathetic) centers, which would partially or completely overcome the inhibitory influence of the carotid sinus and aortic reflexes, resulting in the simultaneous parasympathetic overactivity observed.

The manifestations of increasing cerebral ischemia in man here described correspond to findings in animals. Kisch and Sakai (13), Anrep and Segall (14), without full appreciation of the rôle of the vascular reflexes, concluded that in animals cerebral ischemia produces initial tachycardia and vasoconstriction. Subsequently, the vagus center is reexcited and bradycardia or cardiac arrest follows. Heymans (15) concluded that the vagal centers of the dog are easily stimulated by acute cerebral anoxemia. The responses of the medullary centers of animals of different species, as well as of the same species under different kinds of anesthesia, may show differences (16, 17, 18). Our observations on unanesthetized man in this and in previous studies (19, 20) are interpreted as indicating that under certain physiological stress or pathological conditions partial or general overactivity of both sympathetic and parasympathetic nervous systems may occur simultaneously. Such overactivity is produced by stimulation of peripheral reflexes or of the centers, or of both together.

The vasomotor collapse described by us depended on the combined effect of nitrite and tilting. The condition was precipitated by the orthostatic position of the body, and was promptly abolished by a return to the horizontal position. The fall in venous pressure and the resultant decrease in return of venous blood to the heart in the upright position must be due to a pooling of an appreciable amount of blood somewhere entirely within the vascular bed. Had there been an actual loss of a considerable amount of blood or plasma from within the vascular bed, as has been shown to occur in certain types of circulatory failure, complete recovery within such a short period of time after returning to the prone position could not have been possible. The essential feature of the vasomotor collapse induced by nitrite is, therefore, a disproportion between the circulating blood volume and the volume of the peripheral vascular bed (decrease of the "effective blood volume"). The exact mechanism by which the pooling of blood resulting in this disproportion is brought

about will be discussed in a subsequent report (21).

While the vasomotor collapse here described depended on the orthostatic position, collapse of the circulation can also occur in the horizontal position, provided larger doses of nitrite are administered or a marked susceptibility exists. The latter was the case in a few individuals with arterial hypertension in whom transient collapse and syncope occurred in the horizontal position (1). Severe vasomotor collapse also follows the administration of large doses of nitrite to animals in the horizontal position. The orthostatic position, therefore, merely intensified the type of changes in the vascular system, which would occur even in the horizontal position after the administration of large doses of nitrite.

Prolonged orthostatic position alone, particularly without motion, can produce in certain healthy persons changes similar to those here described. As has been discussed in connection with postural hypotension (22), under certain physiological conditions the postural adaptation of the circulation may gradually become inadequate as standing is maintained (23). In most cases the chief cause is not a fault in the postural vascular reflexes, which are often operating excessively, but a deficient intrinsic vascular tone or poor tone in the skeletal musculature. Turner, Newton and Haynes (24) have pointed out that certain individuals consistently have a tendency to faint after a relatively short standing period. Under certain conditions, the upright posture may also lead to serious consequences (10). It is probable that the collapse studied by us could have resulted in serious sequelae had the subjects not been returned promptly to the horizontal position. That the position of the body also plays a significant rôle in clinical syncope and collapse is attested by bedside observations. These syndromes are frequently precipitated in various diseases when the patient assumes an upright position. Furthermore, manifestations of collapse, which develop in the horizontal position, may promptly subside if the return of blood to the heart and the cerebral ischemia are aided by lowering the head and elevating the lower half of the body ("Trendelenburg position").

There is an apparent similarity between the collapse studied by us and the phenomenon described

as "Sportkrankheit" or "gravity shock" which develops if subjects who have engaged in sudden and strenuous exercise are kept immobile in the upright position. This condition, described by Jokl (25), Weltzien (26) and Mateeff and Petroff (27), has been studied recently by Mateeff (28), who claims that the condition depends on the pooling of the blood in the dilated capillary bed of the legs following exercise.

During the state of collapse the degree of ischemia was not the same in various vascular areas. At a time when the pulse was imperceptible over the radial artery it was still felt over the carotid and femoral arteries. When the maximal flow was greatly diminished in the hand, the degree of ischemia was not always markedly abnormal in the leg. The exact cause of this difference is not clear, though the distance of the area from the heart and the gravity effect must have been factors. It is also of interest that the electrocardiograms failed to reveal any appreciable amount of myocardial anoxemia. The explanation of the latter finding may lie in the possibility that the relative decrease in the coronary circulation was less than that in the extremities, and was not out of proportion to the decrease in the work of the heart, which must have been considerable. This explanation seems to be applicable to similar electrocardiographic findings in the presence of clinical syncope, collapse and shock (18, 19, 29).

The demonstration of severe derangement of the circulation with secondary disturbances of the functions of several organs in healthy subjects with normal cardiac and vascular reserves offers a rational implication for a similar state of affairs in disease, in which there is usually an impaired nervous or cardiovascular reserve. The reason for the progressive downhill course of patients with collapse, unless the etiological factors are eliminated in time, becomes obvious.

#### SUMMARY

1. Sodium nitrite, in an amount which produces no symptoms and only slight if any changes in the circulation in the horizontal position, causes circulatory collapse in the immobile upright position.

2. Symptomatic manifestations of this circulatory collapse, which are identical with those in

disease, have been correlated with the changes in the circulation.

3. Tachycardia, fall in the systolic pressure, small arterial pulse pressure and pulse volume, fall in the venous pressure in the foot, arteriolar constriction with a moderate or pronounced decrease in the "actual" blood flow but with only a small decrease in the "maximal" blood flow in the hands preceded the symptoms of collapse.

4. Pronounced manifestations of circulatory collapse appeared when the maximal blood flow through the hands reached a level of 20 to 40 per cent of the normal value. Simultaneously, there was a fall in the venous pressure, usually reaching a level below that of the right auricle. Further decrease in blood flow resulted in vasovagal syncope.

5. Even in the presence of markedly decreased blood flow during collapse or syncope the complexes of the electrocardiogram revealed but minor changes.

6. Changes in the autonomic nervous system appeared as secondary manifestations to the primary action of nitrite on the peripheral vascular system. It is concluded that the simultaneous overactivity of the sympathetic and parasympathetic autonomic nervous systems arose first through peripheral vascular reflexes and subsequently through medullary ischemia.

This investigation was carried out with the technical assistance of Miss Josephine M. McIntire.

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# THE RÔLE OF THE VENOUS SYSTEM IN CIRCULATORY COLLAPSE INDUCED BY SODIUM NITRITE<sup>1</sup>

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An experimental study of the vasomotor collapse induced by sodium nitrite in normal subjects in the upright position has revealed that the state of collapse is accompanied by a fall in venous pressure and an inadequate return of venous blood to the heart from the lower half of the body (1). As long as the subject remains in the prone position, a small dose of sodium nitrite will produce no symptoms and only slight, if any, measurable circulatory changes in terms of blood pressure, heart rate, arterial blood flow or venous pressure. When the subject is raised from the horizontal to the upright position after the same dose of nitrite, however, he soon develops signs and symptoms of collapse. After nitrite has been given, the peripheral circulation is unable to adjust itself to the strain of the orthostatic position.

Our purpose in the present work was further to clarify the mechanism of the nitrite collapse. On rising to the upright position, the greatest relative change in the circulation is on the venous side of the vascular system in the lower half of the body. Here the venous pressure is normally raised from an average prone value of 5 cm. of water to about 100 cm. of water in the feet and 50 cm. in the abdomen. This consideration, as well as the fact that previous studies revealed no appreciable dilatation of the arterioles or change in the cardiac output after nitrite (2), turned our attention to the venous side of the circulation as possibly one of the most important sites of action of sodium nitrite. We therefore undertook to test the effect of nitrite on the distensibility of the peripheral vascular beds under increases in venous pressure. We were particularly interested to know whether the effect of nitrite on the venous system is generalized or limited to certain vascular areas, such as the splanchnics.

In the present study, the hands of normal sub-

jects were placed under known increases in venous pressure, and the corresponding increases in hand volume were measured both before and after the administration of nitrite. The subject remained in the prone position and in this way other factors, such as changes in arterial blood pressure, pulse or blood flow, were eliminated. Capps, working in this laboratory, developed this method as a means of measuring the tone or "resistance to stretch" of the capillaries, venules and veins of the hands in both normal and diseased states (3). We have used this procedure, with minor modifications, with satisfactory results.

## METHOD

The subject rested in a comfortable horizontal position for at least 45 minutes after all apparatus had been adjusted before any observations were begun, and remained in this position throughout an experiment. Arterial blood pressure was determined in the arm by the usual auscultatory method, using a mercury manometer. The heart rate was counted by arterial palpation or from the plethysmographic tracings. The rate of blood flow was determined by Frecman's modification of the plethysmographic method of Hewlett and Van Zwaluwenburg (4). The averages of from 5 to 10 separate tests were used for each measurement of the blood flow.

After the blood flow had become constant, the tone of capillaries, venules and veins was determined by measuring the increases in volume of the hand when subjected to increases of venous pressure in amounts of 10 mm. Hg up to 50 mm. Hg. This was done by inflating a pressure cuff around the wrist at these pressures. To avoid the possible objections that the resultant changes in volume are due in part to reactive hyperemia, edema formation or spontaneous changes in hand volume, each pressure was done separately, so that the time during which the pressure was applied was only a few seconds and any change in the base line was immediately apparent.

In determining the increments in volume, the hand volume at 10 mm. Hg venous pressure was used as the base, by subtracting the increase due to 10 mm. Hg from that due to 20, 30, 40 or 50 mm. Hg, respectively. This procedure eliminates the effect of any possible spontaneous variation in systemic venous pressure, and also makes all the increments in volume due to equal increments in

<sup>1</sup> This investigation was aided in part by a grant from the Josiah Macy, Jr., Foundation.

venous pressure (10 mm. Hg). The total hand volume was determined by measuring the displacement volume of water in the plethysmograph. From this the volume changes were calculated per liter of hand volume.

The responses of these various functions to the following experimental conditions were determined on each of 6 normal subjects on separate days: (1) Both hands were kept at a temperature of 32° C. (normal hand temperature). After several normal control values had been obtained, the effect of an oral dose of 0.12 to 0.18 gram (2 to 3 grains) of sodium nitrite was measured. (2) Both hands were kept at 37.5° C. (body temperature). After establishing normal values, the effect of the same dose of sodium nitrite was determined. (3) One hand was kept at 32° C., and the other maximally dilated at 45° C. This heat not only caused local dilatation in the hot hand, but also produced reflex vasodilatation with maintained vasomotor reactivity in the cool hand. After constant values had been established, the effect of the same dose of sodium nitrite was measured.

## RESULTS

Figure 1 shows the tracings obtained from a typical subject, S. M. Both hands, at 32° C., were subjected to increases in venous pressure, which caused corresponding increases in hand volume. These increases in volume were remarkably constant on repeated tests under the same conditions. After 0.12 gram (2 grains) of sodium nitrite had been given, however, there was a marked increase in the volume changes produced by the same venous pressures. It is important to note that after nitrite, although there

was a definite decrease in the resistance of the "venous" vessels to stretch, as measured by the height to which the curves rise, there was simul-

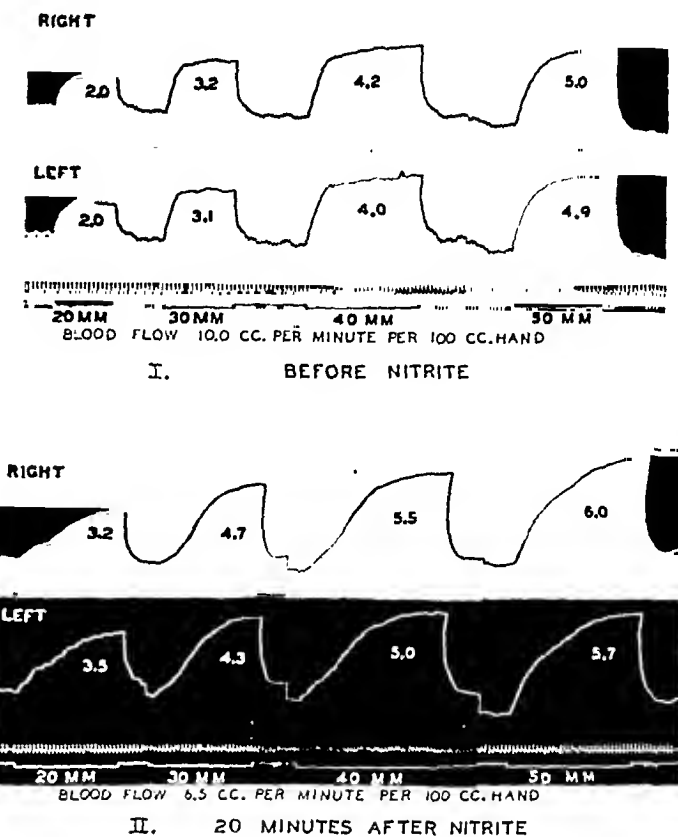


FIG. 1. SUBJECT S. M. PLETHYSMOGRAPHIC RECORDS OF THE VOLUME INCREASES CAUSED BY INCREASES IN VENOUS PRESSURE IN BOTH HANDS AT 32° C., BEFORE AND AFTER THE ADMINISTRATION OF 0.12 GRAM OF SODIUM NITRITE.

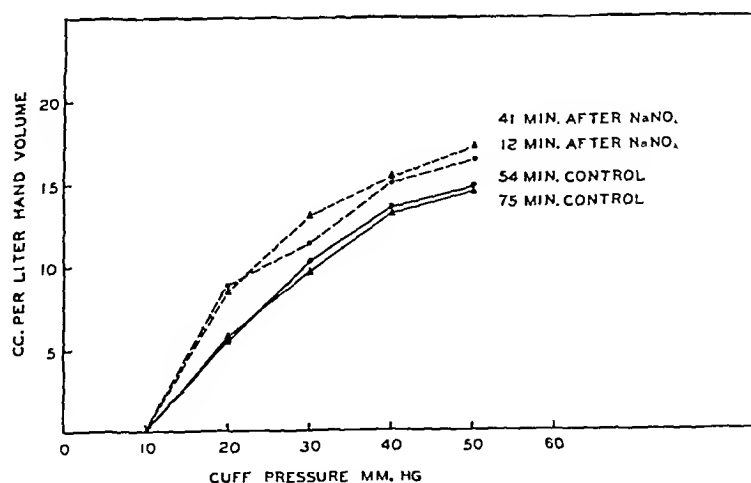


FIG. 2. SUBJECT S. M. VOLUME INCREASES CAUSED BY INCREASES IN VENOUS PRESSURE IN ONE HAND AT 32° C. BEFORE AND AFTER THE ADMINISTRATION OF 0.12 GRAM OF SODIUM NITRITE.

The hand volume at 10 mm. Hg venous pressure was used as a base.

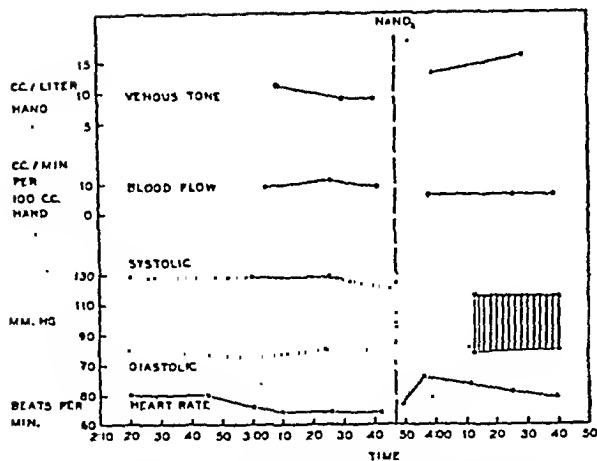


FIG. 3. SUBJECT S. M. EFFECT OF 0.12 GRAM OF SODIUM NITRITE ON THE VENOUS TONE AND THE BLOOD FLOW IN ONE HAND AT 32° C., ON THE ARTERIAL BLOOD PRESSURE AND ON THE HEART RATE.

The venous tone is measured by the increase in hand volume per liter of hand produced by increasing the venous pressure from 10 to 30 mm. Hg.

taneously an increase in the resistance offered by the arterioles to the flow of blood, indicated by the steepness with which the curves rise. Hence, after the administration of nitrite there was within the same vascular area both a decrease in venous tone and at the same time an increase in arteriolar tone. The arteriolar constriction shown in this instance we believe is a response

to and not a result of the nitrite, as it did not occur in every case. An arteriolar dilatation was never demonstrated after nitrite, however; there was either no change or, as in this case, a slight constriction. Therefore, we conclude that the changes produced directly by nitrite must involve elements of the vascular bed peripheral to the arterioles, i.e., capillaries, venules and veins. This is in agreement with Capps' conclusions (3) that his method actually measures the tone of those vessels peripheral to the arterioles, mainly the venules and the veins.

In this subject, therefore, the capillaries, venules and veins of the hand were distended by the same venous pressures from 20 to 30 per cent more after nitrite than before. Figure 2 is a graph of the volume increases caused by increases in venous pressure, before and after the administration of nitrite to the same subject (S. M.).

Figure 3 is a chart of the whole experiment, showing the changes in pulse, blood pressure, blood flow and "venous" tone. For the latter values the increment in hand volume produced by the increment of venous pressure from 10 to 30 mm. Hg has been calculated for each liter of hand volume. This gives a single figure representative of the changes as graphed in Figure 2.

Figure 4 shows the effect of 0.18 gram (3

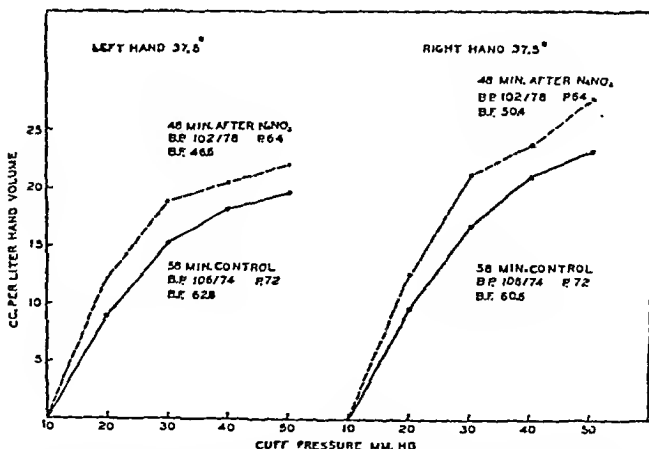


FIG. 4. SUBJECT R. W. VOLUME INCREASES CAUSED BY INCREASES IN THE VENOUS PRESSURE OF THE TWO HANDS AT 37.5° C. BEFORE AND AFTER THE ADMINISTRATION OF 0.18 GRAM OF SODIUM NITRITE.

The hand volume at 10 mm. Hg venous pressure was used as a base.

grains) of sodium nitrite on the tone of the capillaries, venules and veins of the hands of Subject R. W. Both hands were at  $37.5^{\circ}\text{C}$ . It should be noted that although there was a decided decrease in the tone of these vessels (i.e., a "dilatation") after the nitrite, there was little or no change in blood pressure, pulse or blood flow. There were no symptoms. It is of interest that these changes in venous tone were not apparent under the normal low venous pressures of the prone position. The hand volume increased only slightly, if at all, after the administration of nitrite.

After small doses of nitrite, some subjects showed slight or no change in the venous tone of the hand, especially at the lower temperatures, which cause local vasoconstriction. When the hands were already moderately dilated, however, as at body temperature ( $37.5^{\circ}\text{C}$ ), or reflexly dilated by immersing the opposite hand in hot water ( $45^{\circ}\text{C}$ ), the effect of nitrite on the venous tone became more apparent.

Of 6 subjects tested with both hands at  $32^{\circ}\text{C}$ ., only 2 showed a definite decrease in venous tone after nitrite, 2 showed a slight decrease and 2 showed no change. Of 5 subjects tested at  $37.5^{\circ}\text{C}$ ., 4 showed a definite decrease in tone and 1

showed a slight decrease. Of 6 subjects tested with one hand at  $32^{\circ}\text{C}$ ., reflexly dilated by having the other hand at  $45^{\circ}\text{C}$ ., 5 showed a definite loss of tone after nitrite, while 1 showed no change.

Figure 5 is a graph of the changes in tone after the administration of nitrite in Subject T. L. at various temperatures and shows the trend of the results of the whole group. There was no change after nitrite with both hands at  $32^{\circ}\text{C}$ .; a definite decrease in tone after nitrite with both hands at  $37.5^{\circ}\text{C}$ .; and a decrease also with the hand at  $32^{\circ}\text{C}$ ., reflexly dilated by keeping the opposite hand at  $45^{\circ}\text{C}$ .

We have found that at local temperatures above  $40^{\circ}\text{C}$ . this method for measuring the tone of the capillaries, venules and veins in the hand is impractical. At these temperatures there is either no effect or an apparently reverse effect after nitrite. Likewise, at such high temperatures we have found that other procedures, such as elevation of the body to an upright position, which we know should increase the tone of these vessels, apparently result in an increase in distensibility. Measures which cause dilatation, on the other hand, as returning the subject to a horizontal from an upright position, apparently cause a decrease

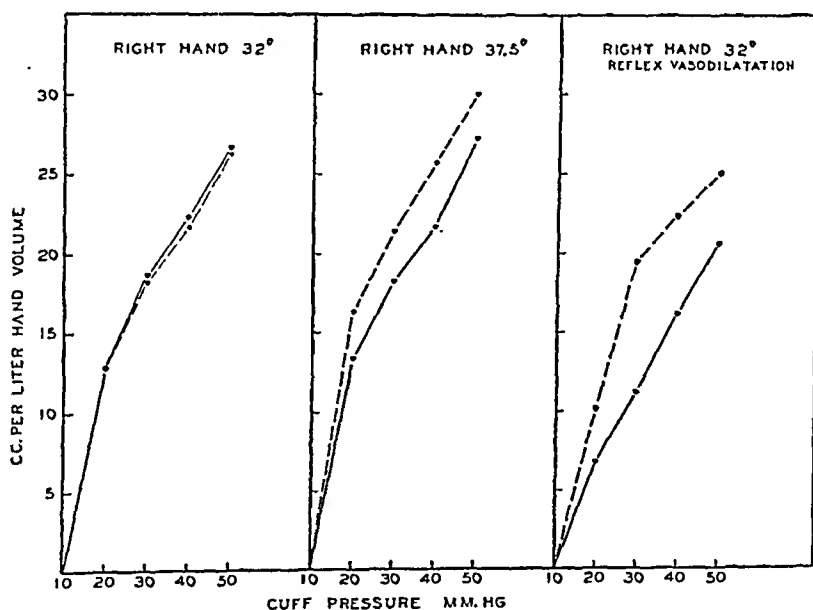


FIG. 5. SUBJECT T. L. VOLUME INCREASES CAUSED BY INCREASES IN THE VENOUS PRESSURE OF THE RIGHT HAND AT  $32^{\circ}\text{C}$ ., AT  $37.5^{\circ}\text{C}$ . AND AT  $32^{\circ}\text{C}$ . REFLEXLY DILATED BY HAVING THE LEFT HAND AT  $45^{\circ}\text{C}$ . BEFORE (SOLID LINE) AND AFTER (BROKEN LINE) THE ADMINISTRATION OF 0.18 GRAM OF SODIUM NITRITE.

in distensibility. This effect is possibly due to the fact that at this high temperature the vascular bed of the hand is already fully dilated and therefore distended against the integument and fascia of the hand. Any further distention is actually against the skin and fascia, and not of the vessels alone. The more completely the vessels are already dilated, the less the hand can be further distended by any means, because of the limiting effect of the inelastic integument. Since the method used depends upon the ability of the vessels to distend freely under increases in venous pressure, it obviously cannot be used in the hand when the vessels are already completely or almost completely dilated by local heat. Except for this objection, the method has given uniformly satisfactory results.

*Vasomotor responses after administration of the nitrites.* It has been shown that the changes in venous tone produced by sodium nitrite are independent of the existing state of the arterioles. It was of interest to ascertain whether under such conditions the responses of the arterioles, as well as of the venules and veins, are influenced by nitrites. We have therefore analyzed the vasomotor effects of two types of stimuli, namely, those of pinch and of increased respiratory movements. The effect of pinch on the vascular system of the normal hand has recently been studied by Capps (5). He has shown that this noxious stimulus produces a sudden and transient decrease in the blood flow due to reflex arteriolar constriction, and, in addition, a reflex contraction of the venous vessels. The changes in these two types of vessels do not necessarily parallel each other. The arteriolar changes are more regular and more intense. Uhlenbruck (6) and Stürup, Bolton, Williams and Carmichael (7) have also demonstrated a reflex decrease in volume of the human arm and finger after deep inspiration, as well as after pain and other stimuli.

Our observations have confirmed the findings of these workers. We also found that these reflex vascular changes caused by pinch and respiration remain qualitatively and apparently quantitatively the same after nitrite as before. The increase in blood flow in response to an elevation of the local temperature, which depends upon the relaxation of the arterioles, is also the same after nitrite as under normal control conditions. These

observations indicate that in spite of the changes in the venous tone following nitrite the vasomotor reactivity of the vessels was essentially maintained.

#### DISCUSSION

The results of the previous experimental study of collapse produced by sodium nitrite suggested that in this type of collapse the essential change is in the peripheral circulation (1). After even small doses of nitrite there is a loss of tone of the peripheral vascular beds so that under the increased venous pressures of the upright position they dilate in the lower half of the body until they hold a considerable portion of the total blood volume. As a result, there develops a disproportion between the total volume of blood and the total volume of the vascular beds, which we believe is the fundamental mechanism of collapse. Large doses of nitrite alone may produce this state without the additional stress of the upright position.

Whatever essential primary action sodium nitrite has on the peripheral circulation must be independent of the position of the subject and should also be present in the prone position. In this study we have demonstrated a specific effect of nitrite on the peripheral "veins" by measuring the increase in volume of the hand due to known increases in venous pressure. We have shown that the hands are distended by the same venous pressures from 20 to 40 per cent more after nitrite than before.

Capps has previously shown that the method measures the tone of the capillaries, venules and veins of the hand. Our results confirm his contention that this test does not measure the tone of the arterioles. Furthermore, we have shown that small doses of nitrite do not essentially affect the tone of the arterioles or their reflex or local vasomotor responses to various types of stimuli. Thus, in the presence of decreased venous tone the reactivity of the arteriolar system is maintained. The fact that arteriolar constriction and "venous" dilatation have been observed simultaneously within such a small localized area as the hand, while the other bodily functions remained in the control state, suggests that simultaneous constriction of the arterioles and dilatation of the venous vessels may occur in collapse



clinically and can develop without the presence of increased epinephrine secretion. The marked arteriolar constriction (as indicated by a very low blood flow) present at the height of the circulatory collapse which we produced experimentally, as well as that which occurs clinically, can be explained as a secondary response to and not the primary essential pathological physiology of collapse. This sharp arteriolar constriction, on the other hand, coupled with the small arterial pressure, may result in such a low blood flow to the tissues that a dangerous degree of anoxemia is produced. When this anoxemia involves the vital centers, such damage may be done to them that a "vicious circle" is established, and the patient goes deeper into collapse and dies.

The demonstration of decreased venous tone in the hand indicates that the venous system of the splanchnic area is not the sole site of the pooling of blood and is not a specific area for a "venous depot" in collapse. In the upright position the arms and hands become such blood depots, as well as the feet, legs and abdominal viscera. In the horizontal position, likewise, all the dependent portions of the body play such a rôle, if the venous tone is decreased. This is borne out amply by clinical observations on patients in collapse.

That the rôle of the spleen and of the splanchnic area as representing "blood depots" has been overestimated is supported by observations on animals. Dale and Richards (8) have noted that the vasodepressor effect of histamine is maintained after animals have been eviscerated. Frey and Kraut (9) have concluded that the vasodepressor effect of certain organic substances depends on vasodilatation in the skin and muscles. Weiss, Robb and Ellis (10) and Wollheim (11) indicated that the subpapillary venous plexuses of the skin under certain conditions represent important blood reservoirs. Hochrein and Keller (12) attributed such a function to the pulmonary veins. Lindgren (13) has attributed an important rôle to vascular changes within the muscles in collapse.

As far as we know, the data here presented represent the first direct evidence that a chemical substance, namely, sodium nitrite, may lead to circulatory collapse by acting primarily on the venous side of the circulation, reducing the tone

of the capillaries, venules and veins. Presumably other vasodilating drugs and toxins may act in a similar way. Histamine has been shown to have a dilator effect on the human venous vessels, and when given in small doses this may be its only action on the vascular system (10). By showing that a loss of venous tone is accompanied by a tendency to collapse, we have established the value of tests which measure the tone of the capillaries, venules and veins in the study of collapse. Postural adaptation tests, since they indirectly measure the "venous tone" in the lower half of the body, are of value (14). The test of venous tone in the hand offers a method likely to prove valuable to clinical investigators. Investigation of the venous side of the circulation will prove fruitful in solving the problems of peripheral circulatory collapse.

#### SUMMARY

1. The vascular tone and vasomotor reactions in the hands of normal subjects have been studied by plethysmographic methods before and after the administration of from 0.12 to 0.18 gram of sodium nitrite.

2. Sodium nitrite decreases the tone of the veins of the hand, as indicated by the decrease in resistance of these vessels to graded pressure. The volume of the hand remains essentially unchanged at a normal level of venous pressure, but it becomes increased from 20 to 40 per cent more after nitrite than before, at elevated venous pressures. The decrease in tone of the veins after nitrite is less at a local temperature of 32° C. than at 37.5° C., or at 32° C. with reflex dilatation.

3. The tone of the arterioles, as indicated by the blood flow, remains normal even in the presence of a pronounced decrease of venous tone.

4. The vasomotor responses of the arterioles and veins to certain local and reflex stimuli are maintained in the presence of decreased venous tone.

5. On the basis of the observations described in this and in the preceding study, it is concluded that the primary action of sodium nitrite is on the venous side of the circulation. This action, under elevated pressure, results in a disproportionate increase in the volume of the venous system, causing peripheral pooling of venous blood and hence collapse.

6. The observations reported represent additional evidence demonstrating that the splanchnic area is not the sole or the specific vascular area functioning as a blood reservoir (depot) in collapse.

7. The studies demonstrate the active rôle of the venous system and gravity in certain types of collapse.

This investigation was carried out with the technical assistance of Miss Josephine M. McIntire.

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# THE OSMOTIC PRESSURE OF PROTEINS IN WHOLE SERUM<sup>1</sup>

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A study has been made of the colloidal osmotic pressure of human sera and pathological accumulation of fluids in an effort to gain information concerning the relations of semipermeable membranes and biological colloids and particularly to determine in serum the osmotic effects of the important colloids, albumin and globulin.

## METHOD

For the sake of economy of material and technical simplicity an osmometer with a flat membrane was selected. For membranes, collodion, prepared according to the method of Pierce (1) was used at first, since the results seemed to be uniform and reproducible. However, this was soon abandoned for cellophane, with which all the measurements here presented were made. This material was used by Govaerts (2) for his original experiments in 1923 and has been shown by Turner (3) to be well adapted to measurements of colloid osmotic pressure. It can be secured in a highly uniform quality, and a single sheet, a meter square, is sufficient for a great number of measurements. It has another property which led to its substitution for collodion. Hitchcock (4) has shown that collodion films absorb protein in definite relation to their permeability. Collodion may also absorb other constituents from solutions. It has been found that HCl is formed when collodion is shaken with solutions of KCl. Smith and Sternberger (5) found that as much as 12 per cent of the calcium of serum was adsorbed by collodion sacs in ultrafiltration experiments. It was demonstrated by preliminary experiments that cellophane does not adsorb protein from serum or diluted serum, a fact which Hitchcock (6) had established for other proteins. The effect of adsorption of protein by the membrane on measurements of osmotic pressure is not known because the osmotic activity of the adsorbed protein has not been determined. Nevertheless, the use of a membrane which does not adsorb protein would seem to be preferable if only because it eliminates one unknown factor. Experiments have not been made to ascertain whether cellophane, like collodion, adsorbs other ions or forms HCl from KCl.

The osmometer finally adopted is illustrated in Figure 1. It is not strictly original in design, but a modification of those described by Govaerts (2), Krogh and Nakazawa (7), Hill (8) and Moore and Roaf (9). It is so

constructed that the fluid under examination comes into contact only with glass, rubber and the membrane. The metal clamping pieces are entirely external, to eliminate electrical charges on metallic ions, which, as Cox and Hyde (10) have shown, may have a definite effect in ultrafiltration.

The osmometer was at first set up in the usual manner, the membrane supported by a hard rubber disc perforated with many fine holes. Against the lower surface of the perforated disc was placed a piece of blotting paper saturated with normal saline. This was separated from the salt solution of the constant temperature water bath by an air bubble. Owing to capillarity set up by the moisture in the holes of the perforated disc and by the surface film of the air bubble, erratic results were obtained. When the manometers were set up with physiological salt solution in the cell and on the blotting paper the pressure on the balancing manometer plus the column of fluid in the capillary tube above the surface of the bath did not equal, as it should, the sum of the rise in the capillary tube plus the height of the air bubble below the manometer. However, when the supporting disc and the air bubble were removed, i.e. when saline was in contact with both sides of the membrane, more nearly theoretical results were obtained. Therefore, the supporting disc was discarded and the manometer set up as it is depicted in the figure, with no air bubble and with blotting paper alone to support the membrane.

The cellophane is first thoroughly washed in saline to swell it and to remove glycerol. It is then cut into discs, each of which is floated in saline between a disc of white blotting paper and a thin rubber washer of the same size. When they are required for use the membranes with washers and blotters are pressed gently between pieces of dry filter paper to remove excess saline, after which they are placed in the osmometer, where they are held by firm pressure from the bottom metal cap. Just enough fluid to be examined is now introduced through the top so that it will overflow when the capillary tube is in place, with care to exclude the formation of bubbles. The capillary tube is added, the top gland nut screwed down, and the capillary tube moved until the meniscus stands about 70 mm. above the body of the instrument. The osmometer is placed in the water bath and connected by heavy-walled rubber tubing to the balancing column of air and water. Low vapor pressure stopcock grease is used to seal joints. The air bubble trapped under the blotting paper is carefully removed.

The meniscus is observed by means of a thermometer reader which is placed on the tube at the original level of the meniscus. Sufficient counter pressure is applied

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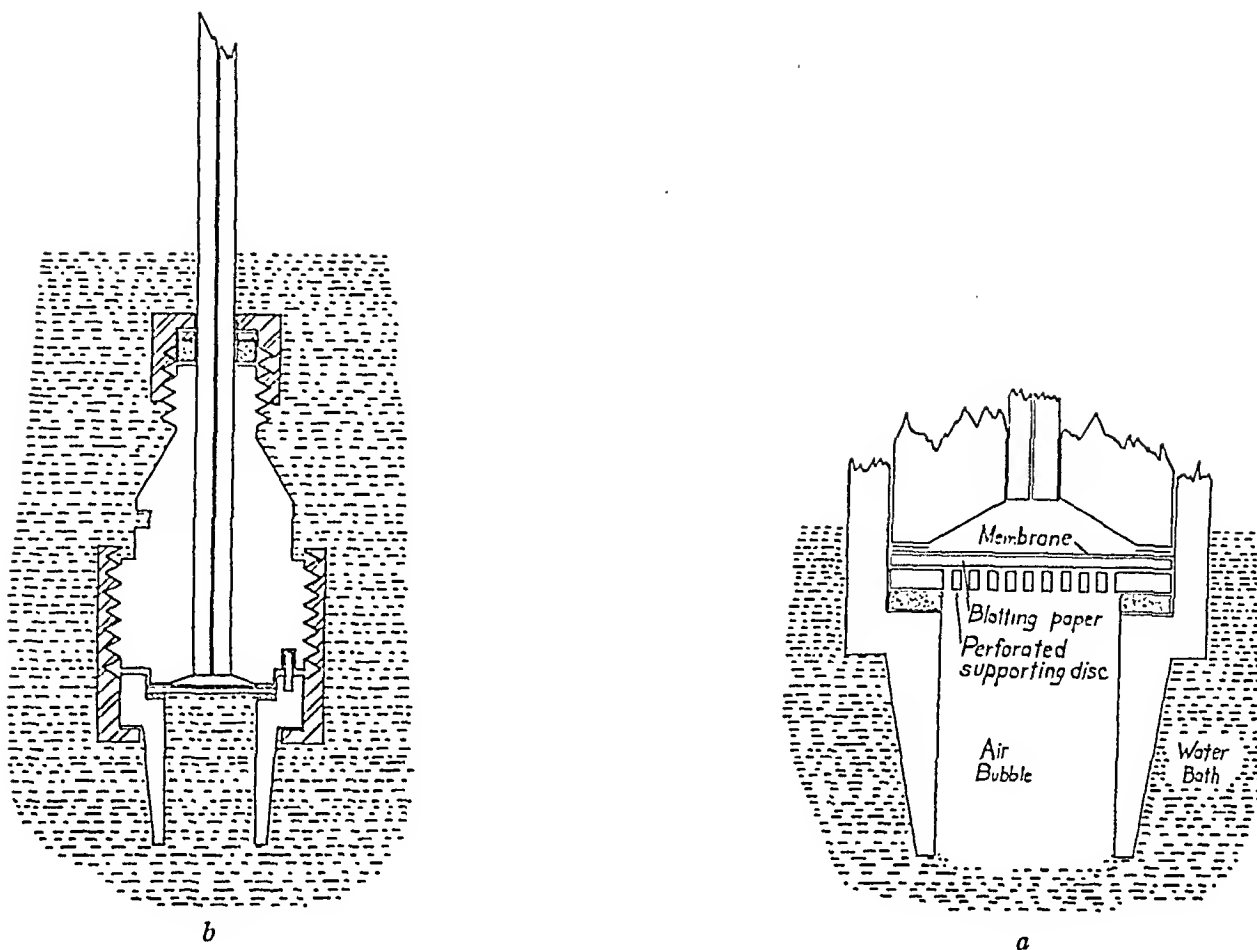


FIG. 1. DETAIL OSMOMETER: (a) AS IT WAS FIRST SET UP; (b) AS IT WAS FINALLY USED.

with a leveling bulb so that the volume of fluid in the osmometer does not change. Frequent adjustments are necessary in the first several hours. The final reading is made at the end of 24 hours. The balancing pressure is measured on a more conveniently located scale against which the leveling bulb is moved.

The temperature of the bath was kept at  $20^{\circ}\text{C.} \pm 0.10$  by means of a toluene-mercury thermoregulator in conjunction with a relay and heating coil, circulation being maintained by a jet of air. Since 0.2 mm. capillary tubing was employed, the volume of serum in the chamber was large in proportion to that in the tubing. Therefore, changes in pressure manifested themselves quickly in movements of the meniscus. The latter was observed with an ordinary thermometer reader, which gave results as precise as the error in the entire method justified. Readings were made at the end of 24 hours and verified after a further interval of 2 hours. If tapping the tube changed the level of the meniscus, another reading was made. It was found that after 24 hours, readings remained constant. These observations were made before the publication of Yanagi (11) who found an apparent change in the colloid osmotic pressure of hypoproteinemic sera after the fifth or sixth hour. This point was not examined specifically. However, there is no evidence, as will be shown later, that the colloid osmotic pressure of sera with low proteins is lower than theoretical considerations demand. Repeated measurements of the capil-

larity of the glass tubes of the osmometers averaged 48 mm. with a variety of sera. Because sufficient material was not always available to permit separate determinations of this correction on each sample of serum, a standard correction of 48 mm. was used. All determinations were made in duplicate, some in triplicate.

Dilutions of sera were made by adding to 1 or more whole cc. of serum the desired amount of physiological saline by drops from a pipette that yielded 30 drops per cc. Sera were not analyzed for protein after dilution.

Serum proteins were determined by Howe's (12) method.

Blood was withdrawn without stasis, and serum was removed with anaerobic precautions (13) to prevent exchanges of water between serum and cells.

In 35 instances, measurements were made on native serum and on the same serum diluted with saline. In 75 of these experiments 2 dilutions were examined, in the other 18 only one. These dilution experiments served a double purpose: they permitted an investigation of the effects of dilution on osmotic pressure and at the same time provided large families of sera containing equal concentrations of either albumin or globulin.

It is difficult with serum to ascertain the actual error of the method because serum cannot be stored for any considerable period and because sera with identical concentrations of protein can not be found. An ampoule of acacia (Lilly's), opened with aseptic precautions, after

dilution with sterile water, was subdivided into 10 cc. lots, each of which was placed in a sterile sealed ampoule. This provided a stock of constant material, 6 per cent acacia in normal saline, that could be used at intervals to check the method. The average reading from 18 different observations on this material was 252 mm. H<sub>2</sub>O, with a standard deviation of 7 and a maximum deviation of 11 mm. These readings agree fairly closely with measurements made by Dr. Abby H. Turner on the same material.

TABLE I

*Differences between duplicate measurements of colloid osmotic pressure of serum*

Range of $\pi$	Number of observations	Average difference	Standard deviation	Average difference
mm. H <sub>2</sub> O		mm. H <sub>2</sub> O	mm. H <sub>2</sub> O	per cent
< 50.....	5	10	11.4	22.5
50 to 100.....	16	6	7.8	6.8
100 to 150.....	34	8	10.4	6.4
150 to 200.....	19	8	9.5	4.6
200 to 250.....	33	11	14.7	4.7
250 to 300.....	24	10	12.5	3.6
300 to 350.....	14	16	19.6	5.2
> 350.....	6	8	11.7	2.1
Total.....	151	7.5	12.5	7

Table I presents statistically the differences between duplicate or triplicate analyses of sera. The errors are approximately the same at all levels of osmotic pressure and therefore relatively larger when the osmotic pressure is small. The probable error can be taken as about 10 mm. of water. This is undesirably large, but seems from experience close to the minimum that can be achieved by clinically practicable techniques. It is evident that measurements on serum are far less reproducible than similar measurements on standard solutions of acacia. This may be partly referable to the fact that the proteins of serum are subject to bacterial and enzymatic decomposition against which no precautions other than chemical cleanliness were taken.

## ANALYSIS OF RESULTS

The experimental data are presented in Table II. In all, there were 173 examinations of 121 sera from 103 subjects, normal adults and patients with a great variety of diseases. The first 51 determinations represent the 17 experiments in which the osmotic pressure was measured in native serum and two dilutions; the next 36, the 18 experiments in which measurements were made in one dilution; the remainder record single observations. In some instances the same subject was examined on more than one occasion. In the statistical analyses described below the third observation (second dilution) on the serum of Subject 12

TABLE II

*Serum protein and colloid osmotic pressure (Bracketed data represent dilutions from single samples of serum)*

Subject	Albumin	Globulin	Osmotic pressure
	per cent	per cent	mm. H <sub>2</sub> O
1	4.00	1.54	230
	3.00	1.16	153
	2.00	0.77	91
2	4.00	2.58	230
	3.00	1.93	148
	2.00	1.29	93
3	3.07	2.00	207
	3.00	1.95	191
	2.00	1.30	122
4	2.44	2.97	166
	1.64	2.00	114
	0.82	1.00	100
5	3.00	1.52	169
	2.00	1.01	109
	1.00	0.51	88
6	3.00	1.98	204
	2.00	1.32	157
	1.00	0.66	94
7	5.00	2.12	303
	4.00	1.70	217
	3.00	1.27	147
8	1.58	2.00	141
	1.00	1.27	73
	0.79	1.00	81
9	4.00	2.49	220
	3.00	1.87	135
	2.00	1.25	112
10	4.31	2.00	269
	3.00	1.40	187
	2.00	0.94	127
11	3.00	2.95	201
	2.00	1.96	134
	1.00	0.98	81
12	3.28	2.35	250
	0.91	0.29	72
	0.89	0.04	69
13	4.00	2.25	277
	3.55	2.00	214
	1.78	1.00	101
14	3.00	3.07	205
	2.00	2.05	115
	1.00	1.02	48
15	4.61	5.10	380
	4.00	4.33	334
	3.00	3.32	262
16	4.00	5.45	330
	3.00	4.08	191
	2.00	2.73	153
17	3.00	3.47	205
	2.00	2.31	109
	1.00	1.16	65
18	3.91	2.55	235
	2.09	1.12	139
	1.86	2.53	96
19	1.00	1.36	35
	4.49	2.36	291
	1.00	0.53	45
20	4.15	2.05	232
	2.07	1.02	124
	4.25	1.95	234
21	2.12	0.98	128
	3.17	4.19	214
	0.99	1.06	80
22	4.27	2.00	221
	2.14	1.00	42

TABLE II—Continued

Subject	Albumin	Globulin	Osmotic pressure
	<i>per cent</i>	<i>per cent</i>	<i>mm. H<sub>2</sub>O</i>
24	3.00	1.88	171
	2.00	1.25	119
25	3.37	2.07	256
	1.61	1.00	106
	2.05	2.37	120
26	1.70	2.00	91
	4.00	2.38	255
27	2.00	1.19	169
	3.00	2.89	192
28	2.00	1.93	113
	4.05	3.52	326
29	2.00	1.74	140
	3.97	2.00	273
30	1.89	1.00	109
	3.35	3.05	219
31	2.20	2.00	119
	3.97	2.07	219
32	1.99	1.04	107
	3.01	1.46	216
33	2.00	0.97	122
	2.70	4.00	191
14	1.36	2.00	82
17	3.52	1.99	221
	3.73	2.37	261
18	1.55	2.34	91
19	4.33	2.00	242
	4.39	2.30	307
	4.72	2.60	332
22	2.90	3.60	228
23	3.70	2.00	172
33	2.45	1.06	206
	3.63	1.88	223
34	4.12	2.50	223
35	4.33	1.64	303
36	3.60	2.82	279
37	4.48	2.54	260
38	4.97	2.10	309
39	4.11	2.08	258
40	3.40	3.22	237
41	5.47	2.57	404
42	4.49	2.37	284
43	4.45	2.28	253
44	2.79	2.57	171
45	4.18	2.00	283
	4.24	2.35	247
	4.48	2.30	307
46	4.00	2.00	191
47	3.00	1.22	139
48	4.53	1.96	245
49	4.20	2.96	300
50	3.21	3.36	225
51	4.42	2.12	324
	4.88	2.43	355
52	2.57	2.23	114
53	4.28	3.92	258
54	4.70	2.29	253
55	2.00	2.06	129
56	3.94	3.13	285
57	4.11	3.50	265
58	3.84	3.02	261
59	3.66	2.55	269
60	1.61	1.00	130
61	3.22	2.00	204
62	3.38	2.09	198
63	3.00	1.62	171
64	5.09	2.36	375
65	3.84	1.92	261

TABLE II—Continued

Subject	Albumin	Globulin	Osmotic pressure
	<i>per cent</i>	<i>per cent</i>	<i>mm. H<sub>2</sub>O</i>
66	5.98	2.60	465
67	3.17	1.96	179
68	4.04	3.44	272
69	5.34	2.80	412
70	4.32	2.00	287
71	4.57	2.37	292
72	4.24	2.09	226
74	2.93	1.67	193
75	1.88	1.66	121
76	4.18	1.89	311
	4.19	2.29	378
77	5.00	2.20	405
78	2.98	1.29	191
	3.14	1.76	213
79	4.15	1.95	291
80	4.37	1.93	302
81	4.80	1.85	325
82	2.29	1.73	197
83	1.85	1.88	128
	2.11	1.59	126
84	2.22	1.75	147
	2.15	1.74	154
85	0.96	2.59	71
86	1.82	4.79	153
87	2.70	2.75	221
88	2.50	9.14	415
89	4.54	2.62	336
90	3.97	5.61	383
91	4.19	4.08	294
92	4.57	2.44	341
93	4.68	2.84	339
94	2.93	8.44	369
95	4.39	3.39	364
96	4.75	2.37	369
97	4.56	4.05	386
98	4.35	2.25	272
99	4.10	4.14	339
100	5.10	2.26	326
101	5.07	1.91	346
102	3.94	3.02	299
103	5.02	2.87	370

was omitted, because the partition of protein is probably erroneous and the concentration of globulin absurdly low.

The following symbols will be used throughout the discussion of the data:

P, A, and G represent grams of protein, albumin and globulin, respectively, in 100 cc. of serum.

$p$ ,  $a$ , and  $g$  represent the osmotic pressures of 1 gram of protein, albumin and globulin, respectively, in 100 cc. of serum.

$\pi$  = the colloid osmotic pressure of serum in mm. H<sub>2</sub>O.

$W$  = the grams of water in 100 cc. of serum:

It has been repeatedly demonstrated that the relation of P to  $\pi$  is curvilinear, that the apparent

osmotic pressure per gram per cent of protein increases with the concentration of protein (7, 14, 15, 16, 17, 18, 19, 20, 21). There is no agreement, however, concerning the nature or magnitude of the curvature. Part of the confusion results from the failure to differentiate the results of statistical treatment of clinical data from the results of dilution and concentration experiments. The former may be dismissed shortly. The curved relation of  $P$  to  $\pi$  in raw material arises chiefly from the fact that low protein in serum is almost always and entirely referable to deficiency of albumin, osmotically the more active of the protein fractions. With the exception of Grönwall (21), those who have centered their attention upon dilution and concentration experiments have found relatively slight curvatures. Even Grönwall finds the ratio  $\pi/P$  reasonably constant within limits of protein concentration found in sera of normal subjects or patients.

Theoretically, the osmotic pressure of the proteins as actually measured includes, in serum, in addition to the true osmotic pressure of the proteins, an increment contributed by the Gibbs-Donnan effect. Because of their large molecular size a small correction must also be made for the volume of the proteins, since osmolar concentration is properly expressed in terms of mols of solute per weight of water in any mixture. The curvature from this source should, if there is no "bound" or non-solvent water, be small.

It was hoped that the dilution experiments would provide a basis for evaluation of the curvature. In the three-point experiments there is, as theory demands, a perceptible tendency for  $\pi/P$  to diminish with  $P$ . There are, however, distinct exceptions to the rule, particularly among sera with originally low concentrations of protein. It has already been mentioned that analytical errors are greater when protein is low. In the two-point experiments there is a similar tendency for  $\pi/P$  to vary with  $P$ . In both series, the variation is so small and so variable that the curvature which it represents cannot be evaluated. It is evident that it is small, but significant. It is quite probable, judging from the investigations of Adair and Robinson (19), Mayrs (15) and others, that even if the analytical methods were greatly refined, a certain amount of variability would persist. The curvature is appreciably diminished,

but not abolished when the concentration of protein is corrected from grams per 100 cc. to grams per kilo of water by the aid of the equation,  $W = 98.40 - 0.718 P$ , developed by Eisenman, Mackenzie and Peters (22).

TABLE III  
*Relation of concentration of total protein,  $P$ , to osmotic pressure  $\pi$*

Number of observations	Range of $P$	Average $P$	Average $\pi$	$\pi/P$	A/G
	<i>per cent</i>	<i>per cent</i>	<i>mm. H<sub>2</sub>O</i>		
20	<3.0	2.14	85.9	40.1	1.44
26	3.00 to 3.99	3.48	121.5	34.9	1.17
24	4.00 to 4.99	4.50	157.4	34.9	1.46
21	5.00 to 5.99	5.56	220.6	39.7	1.53
48	6.00 to 6.99	6.47	257.9	39.8	1.51
21	7.00 to 7.99	7.32	316.0	43.2	1.56
13	>7.99	9.09	366.8	40.4	0.90
Average				39.4	

In spite of the curvature which is demonstrable in the dilution experiments, when all the 173 observations are examined in families (see Table III), the osmotic pressure per gram per cent protein,  $\pi/P$ , proves to be approximately the same in all families, having an average value of 39.4 mm. H<sub>2</sub>O. Since  $P$  represents the sum of the two fractions, A and G, which may have different osmotic effects, statistical deductions from total protein are open to objection. It is impossible to secure sera in which all combinations of A and G appear; sera with albumin greatly increased or globulin much reduced do not seem to exist. It follows that sera with high protein contain excessive proportions of globulin, while those with low protein are particularly deficient in albumin.

It is possible, however, to meet this difficulty in part by examining the effect of variations of albumin or globulin alone in selected data. In Table IV all observations in which G lies between

TABLE IV  
*Relation of concentration of albumin, A, to osmotic pressure,  $\pi$ , when globulin is between 1.5 and 2.5 per cent*

Number of observations	Range of A	Average A	Average G	Average $\pi$	Average $\pi/A$
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>mm. H<sub>2</sub>O</i>	
18	<2.5	1.93	1.97	125.0	64.8
16	2.5 to 3.49	3.07	1.93	187.7	61.1
41	3.50 to 4.49	3.72	1.89	234.7	63.1
13	>4.49	4.85	2.20	326.5	67.3
Average					63.8



1.5 and 2.5 per cent have been arranged in families of  $A$ . Altogether there were 88 such observations. Neither  $G$  nor  $\pi/A$  in this series varies systematically from family to family. The mean value of  $\pi/A$  is 63.8. This would seem to imply that  $\pi$  becomes 0 when  $A=0$ , which could only mean that the osmotic pressure of total protein was a linear function of albumin alone and that the osmotic contribution of globulin was negligible. When all observations are plotted graphically as  $A=x$ ,  $\pi=y$ , the points take a roughly linear arrangement that appears to pass through or near the origin. There is, however, a distinct tendency for points representing sera with unusually high globulin to diverge from the alignment, an indication that globulin has a small, but perceptible osmotic effect.

So small does this effect appear to be that the correlation between  $\pi$  and  $G$  is hardly more than

perceptible. Only when  $G$  is great can its effect be detected even when only observations with  $A$  between 3.5 and 4.5 per cent are analyzed separately.

Since the curvature of the relation of  $P$ ,  $G$  and  $A$  to  $\pi$  is sufficiently small, reasonably accurate prediction formulae might be secured by treating it as a straight line. With this in mind, formulae relating  $P$ ,  $A$ ,  $G$ , and  $A+G$ , respectively to  $\pi$  have been derived by regression equations. Since osmolar concentrations should, properly, be expressed in terms of weight of solute per weight of water, all concentrations of protein and its fractions were first corrected to grams of protein per kilo of water by means of the equation  $W=98.40-0.718 P$ , mentioned above. It has already been stated that this treatment tended to reduce the curvature in the dilution experiments.

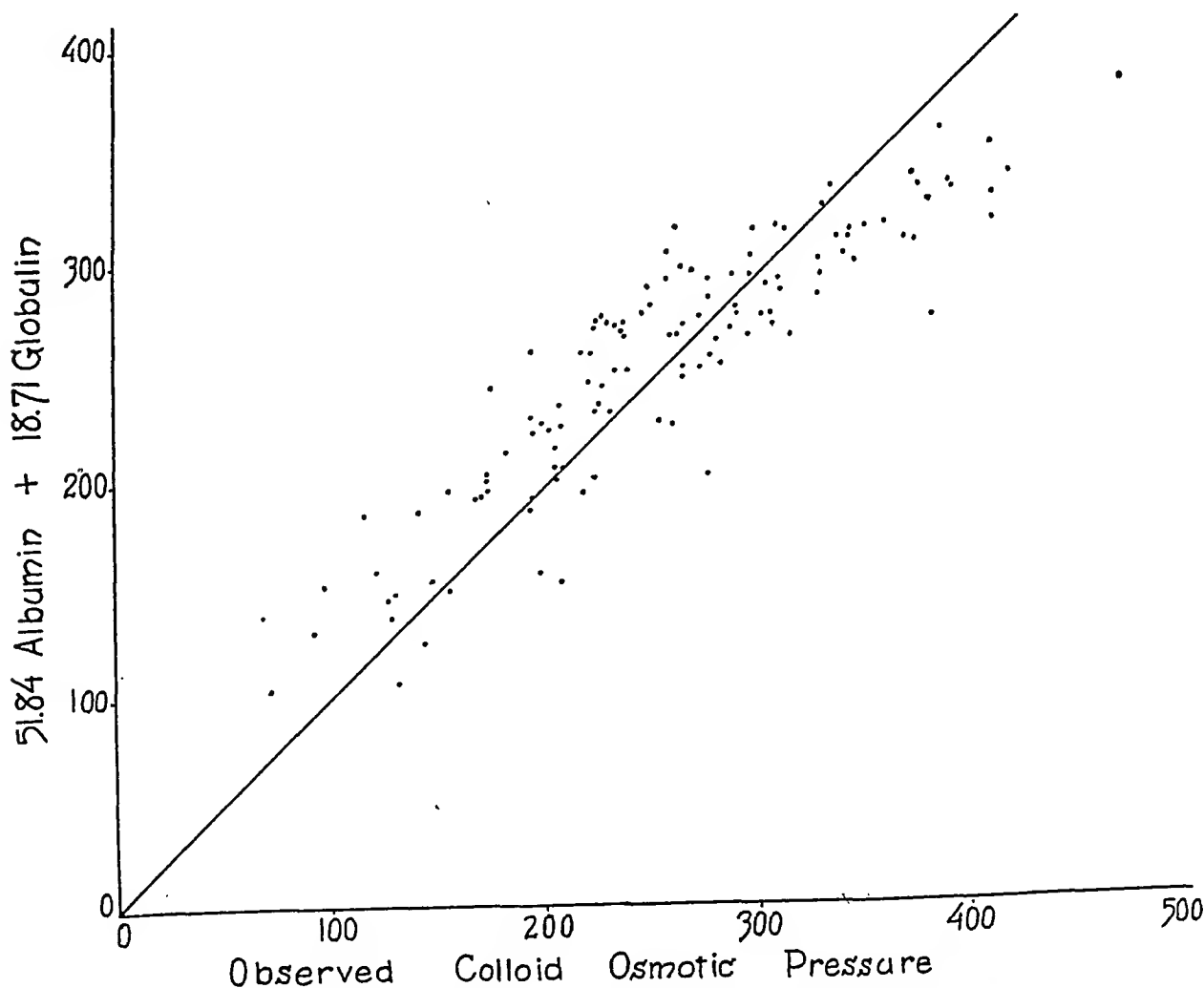


FIG. 2. COMPARISONS OF OBSERVED AND CALCULATED OSMOTIC PRESSURES.  
 $A$  and  $G$  are expressed in terms of grams per 100 grams of water in serum.

The assumption was first made that all curves pass through the origin, i.e., that  $\pi$  becomes 0 when protein does. With this assumption the following equations were derived. In all, protein concentrations are expressed as grams per kilo of water, osmotic pressure as mm.  $H_2O$ . For these estimations, only the 121 measurements on native serum were used. The inclusion of the measurements from diluted sera yields slightly different coefficients, but the differences are not important.

Equation	Average deviation	Standard deviation
1. $\pi = 37.8 P_w$	$\pm 36.7$	$\pm 45.7$
2. $\pi = 64.1 A_w$	$\pm 31.1$	$\pm 44.8$
3. $\pi = 78.9 G_w$	$\pm 90.0$	
4. $\pi = 51.8 A_w + 18.7 G_w$	$\pm 29.7$	$\pm 35.5$

It is obvious at once that the relation of  $\pi$  to G is extremely loose; the average deviation is greater than the coefficient. Formulae derived from P or from A alone are almost equally good, although

the latter is to be preferred slightly, probably because in certain instances the total protein is composed chiefly of globulin, which has a lower osmotic pressure than albumin. The equation derived from A and G together conforms best to the data. The deviations from this equation are presented graphically in Figure 2. The points at the extreme right of the figure fall systematically below the line, while those at the extreme left fall predominantly above it. The points at the extreme right represent observations with unusually high protein composed predominantly of globulin.

It is hard to say to what extent the apparent curvature in the arrangement of the points is comparable to the curvature in the dilution experiments and how far it should be attributed to the combinations of protein fractions in the particular sera which were selected for analysis. The

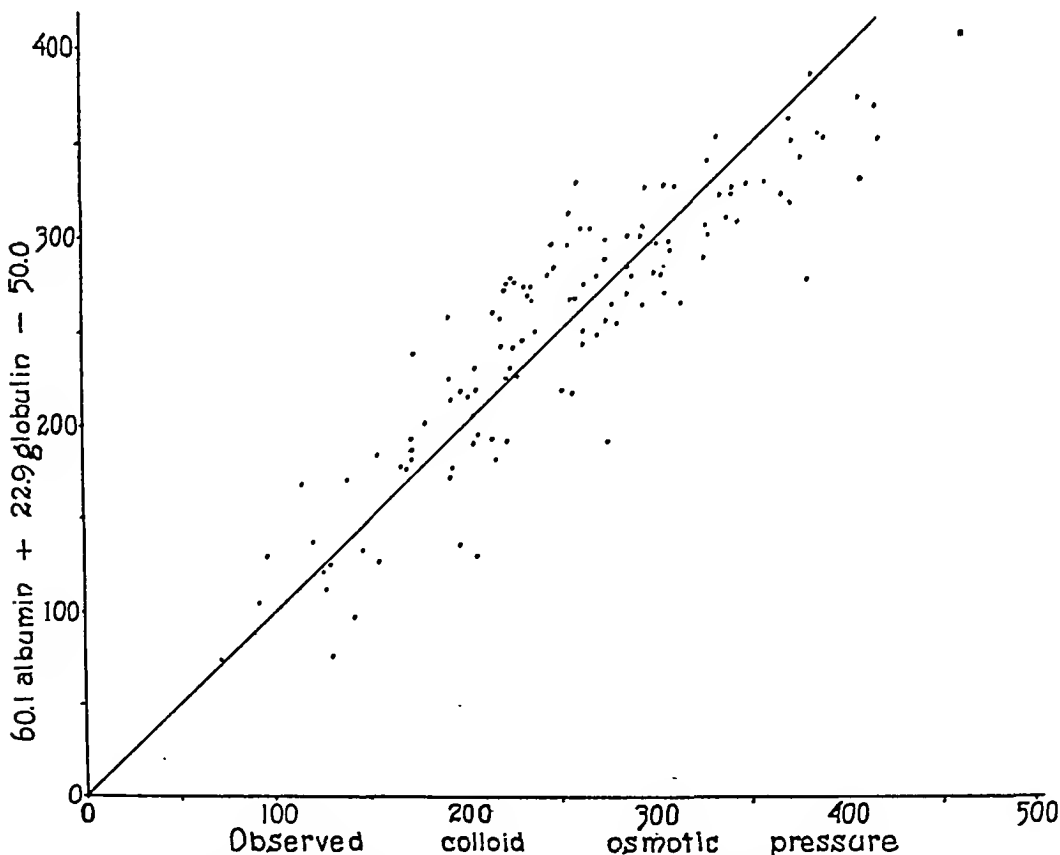


FIG. 3. COMPARISONS OF OBSERVED AND CALCULATED OSMOTIC PRESSURES. A and G are expressed in terms of grams per 100 grams of water in serum.

range of variation of protein concentration could not have been made so large without purposeful selection of sera with abnormal A/G ratios. Since a certain amount of uncorrected curvature may be expected on theoretical grounds, the assumption that the best straight line will pass through the origin is unwarranted. A better fit is obtained by the equation.

$$5. \pi = 60.9 A_{10} + 22.9 G_{10} - 50.0 \quad \text{A.D.} = \pm 27.6 \quad \text{S.D.} = \pm 32.6.$$

This is illustrated in Figure 3.

#### DISCUSSION

Beyond the statement that these measurements of total colloid osmotic pressure agree in general with those of other reliable investigators (7, 14, 15, 17, 20, 23, 24), comparisons on the basis of magnitude alone can not be profitable, since small variations depend so largely on details of technical procedure. The method employed is believed, for reasons stated above, to be soundly devised, and yields reproducible measurements. The errors of measurement are unfortunately large; just how large it is hard to say. It is believed, however, that neither the differences between duplicates in Table I nor the variations of measurements of the osmotic pressure of standard acacia solutions give a correct estimate of the absolute error in actual analyses of sera. This impression is derived from the variability of the dilution curves and also from erratic changes of the protein osmotic pressure in repeated observations on individual subjects. In the majority of instances both the absolute magnitude of the osmotic pressure and the changes of pressure in such cases agree reasonably well with the protein concentrations. Occasionally, however, unaccountable departures from the common rule are encountered. For example, in Subject 19 the change of  $\pi$  between the first 2 observations is altogether too large. It seems likely that the first measurement of  $\pi$  was too small. Similarly, the second  $\pi$  in Case 45 is out of line with the other 2, which are of the expected order of magnitude. Discrepancies of this kind noted by other observers have been attributed to variations in the osmotic properties of the serum proteins without sufficient

consideration of the degree of error in measurement of osmotic pressure. Serum contains no known colloids other than protein which produce an appreciable osmotic pressure. Fishberg (18) has suggested that the lipoids of serum must be given consideration. Adair and Robinson (19), however, found that removal of lipoids had no effect on the osmotic pressure of the serum proteins. Several of the patients in the present study had lipemia, in one or two cases of an extreme degree. Nevertheless, the colloid osmotic pressure of their sera was not unexpectedly high (see, for example, Number 78).

The factors for the osmotic pressure of the separate components of serum, albumin and globulin, in Equation 4, approach the relative orders of magnitude derived by comparable methods by Govaerts (23) and by von Farkas (24). They are not proportional to the generally accepted molecular weights of albumin and globulin; the coefficient of  $G$  is too small and that of albumin proportionately too large. This does not mean that the osmotic pressure of either of the components is necessarily different in serum than it is in pure solution. These equations were developed on a purely empirical basis to fit certain experimental data. Undoubtedly, it would be possible by mathematical juggling to devise a more complicated equation which would fit the data even better than the formulae which have been presented. No significance could be attached to the particular form taken by such an equation. Wells, Youmans and Miller (20) found that data from a series of determinations comparable to those here presented were best described by the equation,  $\pi = P (21.4 + 5.9 A)$ . As they have pointed out, this formula implies that the partial osmotic pressure of globulin depends upon the concentration of albumin in serum. The only evidence for such an interrelation is found in certain preliminary observations by McFarlane (25). Adair and Robinson (19), from similar experiments, concluded that the proteins in serum conformed to Dalton's law of partial pressures. The present measurements differ systematically from those of Wells, Youmans and Miller (20), probably because of certain differences of technical procedure. When allowance is made for this difference, there is still no evidence

that globulin surrenders its individuality when it is combined with albumin in serum. With the variable curvature of  $\pi/P$  with  $P$ , the relatively small osmotic pressure of  $G$  and special characteristics of the particular sera selected for examination,<sup>3</sup> it is idle to draw inferences concerning the specific osmotic properties of protein fractions from statistical treatment of measurements of osmotic pressure in whole serum.

Since the relation between  $\pi$  and  $P$  is curvilinear, the mean line which will best describe it should not pass through the origin. To this extent Equation 5 may be more accurate than 4. In the last analysis, the utility of prediction formulae developed statistically depends on the character of the data on which they are based. The observations here employed are fairly representative and as evenly distributed over a wide range of protein concentrations as clinical material permits. Unless practicable methods for the measurement of the colloid osmotic pressure of serum can be greatly improved in accuracy, estimations from prediction equations based on representative data secured by sound methods may be quite as reliable, for relative purposes, at least, as direct measurements.

#### SUMMARY

The relation of the concentration of serum protein, and its fractions albumin and globulin, to the colloid osmotic pressure of human serum has been reexamined. Empirical equations have been derived for the estimation of colloid osmotic pressure from the concentrations of protein, albumin and globulin in the water of serum.

The authors wish to express their grateful appreciation of aid received from Dr. Alexander Winkler in the statistical treatment of the data and from Dr. Michael J. Lepore who made some of the analyses for protein and measurements of osmotic pressure.

<sup>3</sup> There can be no certainty that the osmotic pressure of the globulin (presumably chiefly euglobulin) which makes up the bulk of the protein in myeloma and lymphogranuloma, the chief sources of hyperproteinemic sera, is the same as the osmotic pressure of normal serum globulin. Failure to include sera with high globulin would limit the practical utility of any prediction formula.

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# HYPERPARATHYROIDISM IN KIDNEY DISEASE

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Extensive investigations of the parathyroid glands were instigated by the cure of generalized osteitis fibrosa cystica by surgical removal of a parathyroid adenoma by Mandl (1) in 1925. It was early noted that renal calculi were often found in cases of parathyroid tumor (2). Later work showed that these stones consisted chiefly of calcium phosphate and that the tendency to their formation was definitely decreased when the urine was made more acid (3). Removal of the parathyroid tumor resulted in the disappearance of this tendency to calculus formation and of the alkalinity of the urine.

Albright, Baird, Cope, and Bloomberg (4), in their discussion of 83 cases of parathyroid tumor with hyperparathyroidism, brought out the fact that 43 of these showed clinical evidence of renal damage. These kidney lesions were attributed to damage resulting from the deposition of calcium in the renal parenchyma or to the formation of calculi in the pelvis, such calcium depositions being manifestations of primary hyperparathyroidism. However, these authors suggested the possibility that parathyroid enlargement may be secondary to renal impairment: "It seems conceivable therefore that a chronic renal insufficiency with phosphate retention and a high inorganic phosphorus level might likewise cause hyperplasia of all parathyroid tissue which might go on to multiple tumor formation" and later, "In these cases the kidney damage may be the cause and not the result of the parathyroid tumors."

Bergstrand (5) studied the parathyroid glands in an extensive series of autopsies, and in most of the cases where he found them enlarged, there was also some damage to the kidneys. Pappenheimer and Wilens (6) carefully weighed the parathyroid glands from a large series of necropsies. They found the parathyroids taken from unselected nephritics to average more than 50 per cent greater in weight than those taken from non-nephritic controls. Seven severe nephritics

showed an increase of 109 per cent over the same control series. The enlargement was diffusely hyperplastic in character, resembling the enlargement which has been reported in rickets (7, 8), and which may exist in pregnancy (9, 10). Adenomata were found in only three of 56 nephritics and in two of 74 controls. Jarrett, Peters, and Pappenheimer (11) report that they have induced a similar enlargement of the parathyroids by producing chronic renal insufficiency in rats.

In previous reports from this laboratory evidence of functional hyperactivity of the parathyroid glands in rickets (12) and in pregnancy (13) has been presented. We have used the same approach in our investigation of parathyroid activity in the present series of nephritic patients,<sup>1</sup> to determine whether the hyperplasia noted by Pappenheimer and Wilens (6) is accompanied by a hyperfunction. The patients were selected on the basis of elevation of the blood urea nitrogen, most of them having chronic rather than acute renal insufficiency. The controls were thirty-eight normal persons—thirty women and eight men. Determinations of calcium and phosphorus were usually done on the same samples of blood which were used for the experimental work.

## METHODS

Serum calcium was determined by the method of Fiske and Logan (14). Serum phosphorus was determined by the method of Fiske and Subbarow (15). The method of Hamilton and Schwartz (16), slightly modified (17), was used to detect hyperactivity of the parathyroid glands. This method consists of measuring the rise of the serum calcium of a rabbit into which 30 cc. of the patient's blood have been injected intramuscularly. Calcium chloride is given the rabbit by stomach tube immediately after injection and one, three, and five hours later. Blood is drawn from

<sup>1</sup> The authors gratefully acknowledge the cooperation of Dr. Andrew J. Brislen, Dr. Edward J. Stieglitz, and Dr. Louis Leiter in obtaining material for this study.

the rabbit before beginning the test and seven minutes after each of the last two administrations of calcium chloride. A rise of 0.30 mM. calcium or more per liter of rabbit serum is taken as indicative of an abnormally large amount of parathyroid hormone in the injected blood.

### RESULTS

Blood from each of 23 nephritic patients was injected into rabbits, and the rise of the serum calcium of the rabbits was determined. The blood of all but three of these 23 patients produced a rise of more than 0.30 mM. calcium per liter of rabbit serum, which indicates that more than normal amounts of parathyroid hormone were present in the injected blood. The results of these parathyroid function tests are shown in Table I, together with the other data on the com-

TABLE I  
*Parathyroid function tests, blood constituents and arterial tensions*

Case number	Parathyroid function test rise	Blood urea	Creatinine	Calcium	Phosphorus	Plasma proteins	Albumin Globulin	Urea clearance (standard)	Arterial tension
	mM. per liter rabbit serum	nitrogen mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	grams per 100 cc.			mm. Hg
1	0.30	122.0	5.6	10.2	4.8	6.31	0.81		228/154
	0.65			10.3	5.4				
2	0.35	73.7	5.1	10.3	5.5	5.93	1.65	3.0	200/116
	0.35			10.1					
3	0.50	61.1	3.0	11.0	3.1	6.78	0.85	5.0	168/90
4	0.35	42.7	1.9	9.8	4.2	3.50	0.48	17.0	148/88
5	0.50	138.7	5.8	8.4	18.8	5.57	1.34	1.9	168/118
6	0.00	92.9		4.3	16.6	5.28	0.71	2.0	135/90
7	0.35	130.0		10.0	4.1	6.73	1.15	2.1	260/150
8	0.50	48.9	2.2		5.8	8.08	1.49	9.0	230/144
9	0.37	15.0		9.8	5.2				280/170
10	0.45	122.0	12.0	9.8	7.1				170/105
11	0.88	40.0	2.5	9.1	10.0				155/90
12	0.45	41.0	2.45	10.2	7.1				220/154
13	0.25	233.0	14.5	8.9	4.4				220/130
14	0.47	35.0	2.5	9.2	6.7				
15	0.00	18.7		8.0	4.3				
16	0.55	40.0	2.5						
17	0.85	94.9		9.0	10.6	5.60	1.38	3.3	154/68
18	0.35	41.5	2.6	9.8					260/140
19	0.38	168.0	8.0	9.0	9.4				110/90
20	0.40	53.0	3.3	10.4	2.7				140/90
21	0.50	49.0	2.6	9.0	3.8				90/07
22	0.67	87.0	8.3	9.2	8.0				220/110
23	0.33	70.0	6.0	11.0	6.7				170/140

position of the blood and on the arterial tension. Where more than one determination of a blood constituent was made, that which was done at the date nearest to the performing of the parathyroid function test was tabulated. Of the 38 controls all but three showed rises of less than 0.10 mM.

calcium per liter of rabbit serum, and none was higher than 0.23. A comparison of the normal and the nephritic series is made in Table II. It

TABLE II  
*Parathyroid function test in normal persons and in nephritic patients*

Rise in rabbit serum calcium	Normals—number of persons	Nephritics—number of patients
mM. per liter		
0.00 to 0.09.....	35	2
0.10 to 0.19.....	1	
0.20 to 0.29.....	2	1
0.30 to 0.39.....		10
0.40 to 0.49.....		4
0.50 to 0.59.....		5
0.60 and up.....		4
	38	26*

\* Three tests were performed on Case 1 and two on Case 2.

is obvious from the figures in Table I that there is no direct correlation between the degree of hyperactivity of the parathyroid glands, as measured by this procedure, and the amount of elevation of the serum phosphorus; nor is there a direct relationship between the parathyroid activity and the blood urea nitrogen. However, the correlation between increased parathyroid activity and chronic nephritis is clear cut.

In order to exclude the possibility that some of the substances which are present in the blood of uremic patients in more than normal amounts were the direct cause of the effect on the blood calcium of the rabbit, 30 cc. of a solution containing the following substances were injected intramuscularly into each of four rabbits: urea, 200 mgm. per 100 cc.; uric acid, 10 mgm. per 100 cc.; creatinine, 5 mgm. per 100 cc.; sodium carbonate, 5 mgm. per 100 cc.; cystine, 10 mgm. per 100 cc.; sodium chloride, 900 mgm. per 100 cc. The same technique was followed as in the tests for parathyroid hormone. None of these rabbits showed a positive test.

### DISCUSSION

As has been suggested by Pappenheimer and Wilens (6), "It is probable that the cases with severe clinical nephritis had phosphate retention, and since any increase in  $\text{PO}_4$  ions will decrease the amount of Ca ions in the blood (Thomson

and Collip (18)) this may incite the parathyroids to increased activity and overgrowth." Since parathyroid hormone tends to elevate blood calcium, hyperfunction of the parathyroids in this instance would constitute a defense against hypocalcemia.

We have shown that there is no proportionality between the increase in parathyroid function and the rise of the inorganic phosphorus of the serum in nephritis; but inasmuch as the phosphorus was generally elevated in the cases which showed increased parathyroid function, our data are not in discord with the theory proposed by the above authors. However, one patient in our series (Case 6, Table I) had developed high arterial tension, albuminuria, and convulsions during pregnancy one and one-half years before the last admission, and had delivered a stillborn child. On the last admission she showed no evidence of parathyroid hyperactivity; the serum phosphorus was extremely high; and the serum calcium in this case alone was below the tetany level. Two weeks after admission in uremia she developed convulsions and died a week later, showing signs of pulmonary edema and shock. Here we may assume that the lack of compensatory parathyroid hyperfunction allowed a profound imbalance of the calcium metabolism.

The failure to elicit an elevation of the rabbits' blood calcium by the injection of the solution containing certain constituents of uremic blood indicated that these substances in uremic blood are not responsible for the reaction. This experiment, however, does not in any way eliminate the possibility that other substances in uremic blood are responsible for the rise.

We agree with the hypothesis of Pappenheimer and Wilens that the hyperphosphatemia of renal insufficiency is the initiating factor in the chain of events which leads to parathyroid hyperplasia. We have shown, moreover, that the hyperplasia demonstrated by the above authors is accompanied by hyperfunction. It is conceivable that this increase in parathyroid activity constitutes a biological defense against impending hypocalcemia secondary to phosphate retention.

#### CONCLUSION

There is increased activity of the parathyroid glands in chronic renal disease, as measured by the method of Hamilton and Schwartz (16).

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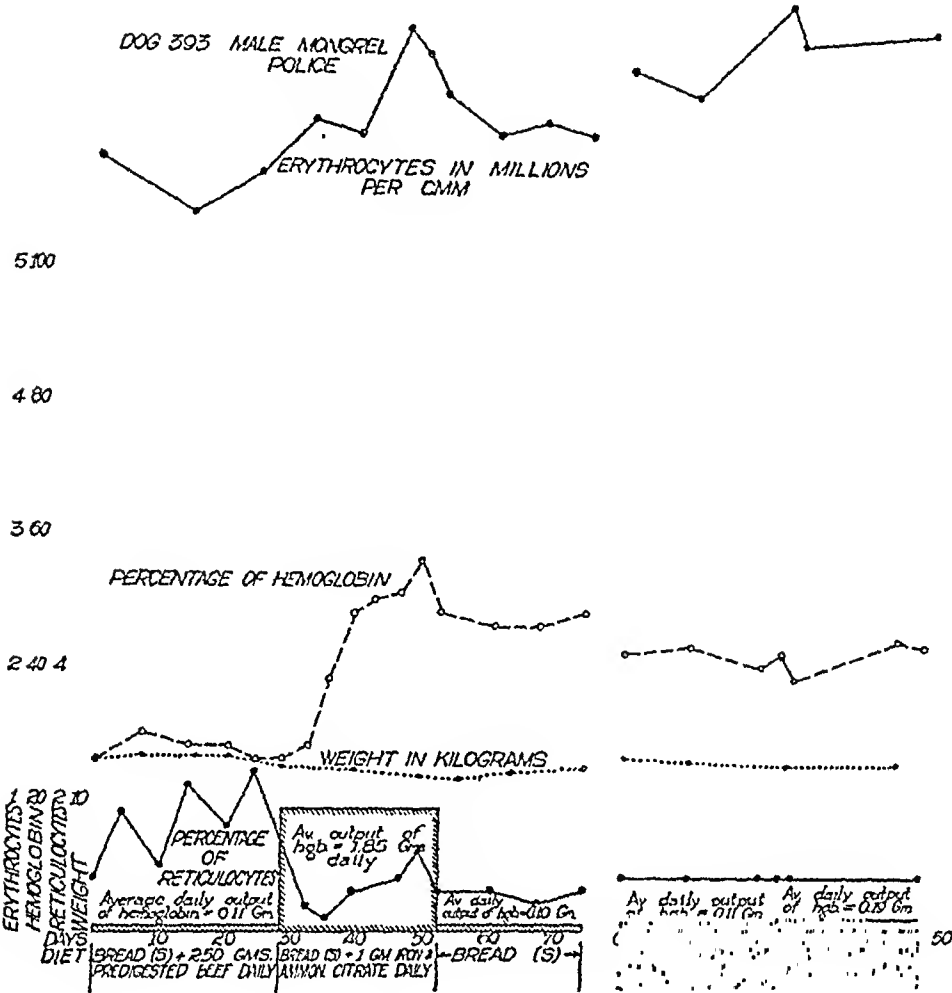


FIG. 1. THE EFFECT OF PREDIGESTED BEEF, OF IRON AND OF LIVER EXTRACT ON THE PRODUCTION OF HEMOGLOBIN AND RED BLOOD CELLS IN A GASTRECTOMIZED DOG.

It is to be noted in Figure 1 that one animal was given predigested beef during two separate periods of study; and, as is shown in Figure 2, the other dog received the meal during one period only. It soon became apparent that the meal was not a palatable one; even when mixed with the standard bread ration the animals refused to eat more than one-third of their allotted portions. Feeding of the meal in small divided portions through a rubber tube passed into the upper intestinal tract was then attempted, but this procedure was invariably followed by regurgitation of most of the mixture. For another period of feeding, the beef was digested with gastric juice removed from the stomach of normal dogs but this method met with no greater success. From the results reported in Figures 1 and 2 and Table I, it may be seen that hemoglobin production showed no increase, while the animals were

TABLE I  
Effect of standard bread (S), of predigested beef, of iron and ammonium citrate and of liver extract on production of hemoglobin in gastrectomized dogs

Dog number	Number of days	Hemo-globin output	Diet and type of therapy
473	20	0.20	Bread (S)
	20	0.24	750 grams predigested beef
	20	1.73	Bread (S) + 1 gram of iron and ammonium citrate daily
	24	0.60	Liver extract, 5 cc. 3 times a week *
394	20	0.04	Bread (S)
	20	2.77	Bread (S) + 1 gram of iron and ammonium citrate daily
	20	0.04	Bread (S) + 5 cc. liver extract daily
393	20	0.10	Bread (S)
	20	0.11	250 grams of predigested beef
	20	1.85	Bread (S) + 1 gram of iron and ammonium citrate daily
	20	0.19	Bread (S) + 5 cc. liver extract daily

\* The authors wish to thank Eli Lilly and Company for their generous contribution of Liver Extract 343 (N.N.R.).

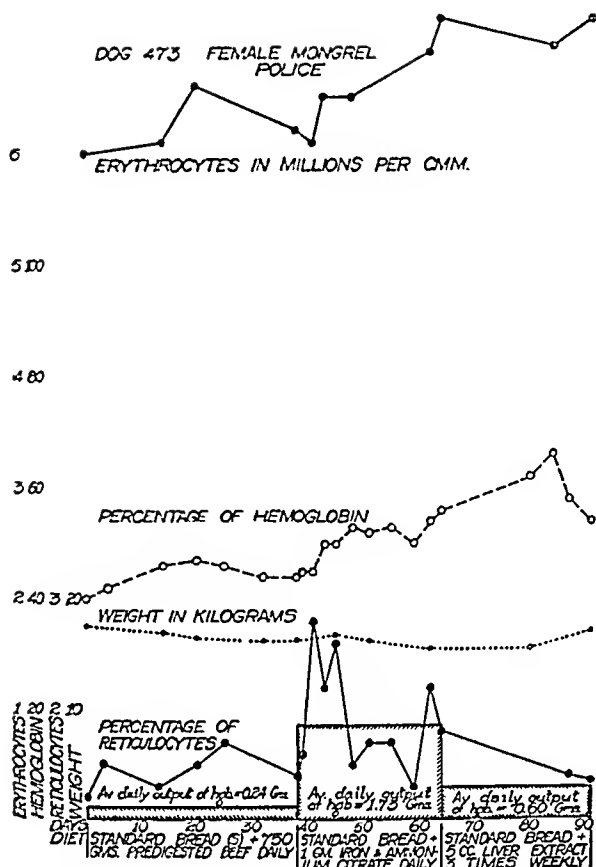


FIG. 2. SHOWS A MARKEDLY INCREASED OUTPUT OF HEMOGLOBIN ON IRON MEDICATION BUT FAILURE TO STIMULATE FORMATION OF BLOOD ON A PREDIGESTED MEAL.

on this regime, over their output during the feeding of the standard bread ration alone. Presumably these results were due largely to the failure of the animals to retain the entire meal.

### 2. The effect of iron and ammonium citrate on the production of hemoglobin.

As a supplement to the standard bread ration, a capsule containing one gram of iron and ammonium citrate (U.S.P.) was given daily to the same three gastrectomized dogs. Prior to medication, the hemoglobin output of each animal had been less than 0.25 gram daily, whereas during the following 21-day period it was 1.85, 1.73 and 1.75 grams respectively. In the third dog (Figure 3), during a later period of iron administration, there was a greatly increased output of hemoglobin up to 2.77 grams daily. Accordingly, the average daily output for all the dogs was 2.03

grams of hemoglobin. These results are slightly in excess of those obtained by others (5) in gastrectomized dogs. On the other hand, they are only about two-thirds of the daily hemoglobin output found by Robscheit-Robbins and Whipple (6) after the administration of iron to healthy dogs in which an hypochromic anemia had been induced.

Figures 2 and 3 show the hematopoietic reaction of the gastrectomized animals to iron as indicated by the reticulocyte response. In the figures it is to be noted that, after 4 days on iron therapy, the reticulocytes rose from a level of approximately 1 per cent to a peak of 3.5 per cent. This maximum, although small, must be considered to represent definite response, when contrasted with the nearly stationary level of 1 per cent which had been observed previously in these animals over a period of weeks. Furthermore, after this rise the reticulocytes dropped to their former level. In view of the microcytic nature of the anemia, with a relatively high red blood cell count, a minimal reticulocyte response is to be expected. Coincidentally with this response, there was an increase in the total number of circulating red blood cells.

### 3. The effect of liver extract (specific for Addisonian anemia) on the production of hemoglobin.

The same three gastrectomized dogs were maintained in an anemic state by bleeding while on the standard bread ration. Each animal was given 5 cc. of liver extract (Lilly's) parenterally three times a week. Figures 1, 2 and 3 show that the hemoglobin output was not increased. Furthermore, there was a lack of response of the reticulocytes. In each animal, however, there was noted a slight but gradual rise in the total number of erythrocytes similar to the results obtained by Ivy and Mullenix and their associates (7, 5).

## DISCUSSION

In undertaking the first experiment recorded here, an attempt was made to obtain by experiments with laboratory animals further information based on previous observations concerning the relationship between gastric digestion and anemia. It was thought that, since an experimentally induced anemia persists in dogs with an artificially produced achylia gastrica, hemoglobin

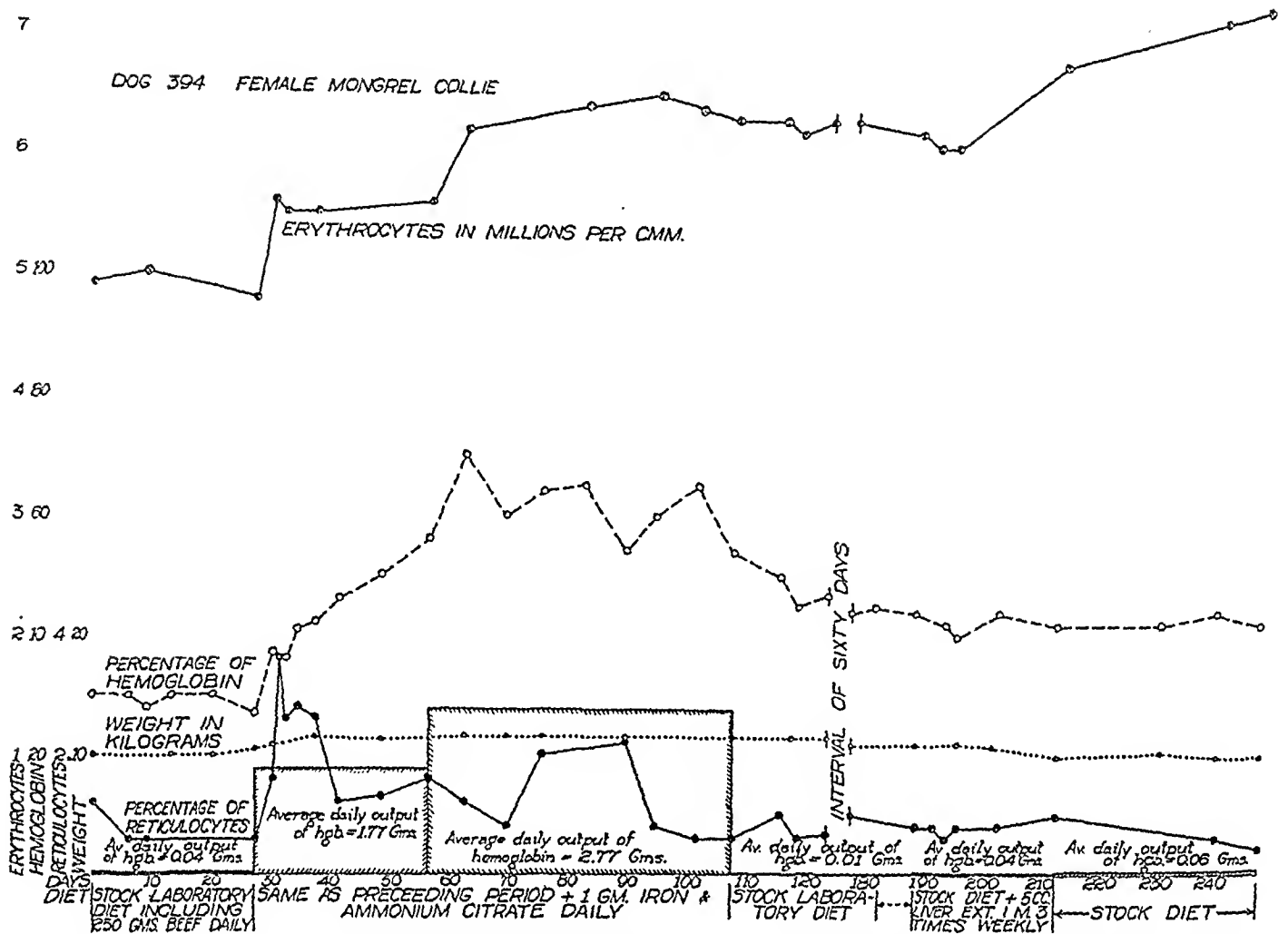


FIG. 3. SHOWS A MARKEDLY INCREASED OUTPUT OF HEMOGLOBIN ON IRON MEDICATION BUT NEGATIVE RESULTS WITH PREDIGESTED BEEF.

Note the gradual rise of erythrocytes during the injections of liver extract.

output could be augmented by feeding a predigested meal. Accordingly, such an experiment was undertaken; but the dogs were reluctant to eat this mixture voluntarily and did not entirely retain it when it was administered through a tube passed into the upper intestines. Our observations were considerably limited by this handicap, and the experiment is therefore inconclusive in its results.

It is clear from the results recorded in the figures that the animals responded favorably to the large doses of iron. The metal used apparently met the demand for replacement of the iron shortage in the body induced by previous bleeding and the artificially produced achylia gastrica. This is in striking contrast to the failure of the gastrectomized dog to obtain from beef sufficient dietary iron to replace his hemoglobin stores as previously reported (1, 2). The experiment

lends further support to the hypothesis that under conditions of depletion of iron in the body, a state of anemia will persist in the presence of an achlorhydria, but may be favorably influenced by iron medication.

The data obtained from the hematocrit determinations indicate that the type of anemia encountered in gastrectomized dogs is decidedly microcytic and hypochromic. This substantiates the report of Mullenix and his associates. In addition, the failure of liver extract to induce a reticulocyte response or materially to increase the hemoglobin output speaks against the tendency of gastrectomized dogs to develop pernicious anemia.

#### SUMMARY AND CONCLUSIONS

1. The effect of feeding predigested beef, of inorganic iron and injections of liver extract on the production of hemoglobin and red blood cells was

observed in three dogs with an artificially produced achylia gastrica. Prior to this study the dogs had been rendered anemic by repeated bleedings, and the hemoglobin output maintained at a low level on a standard bread ration; subsequently, gastrectomy had been performed.

2. Hemoglobin output was not increased while the dogs were given predigested beef, presumably due to a failure on the part of the animals to retain the meal.

3. It was shown that the average daily output of hemoglobin was 2.03 grams, while the dogs were given iron and ammonium citrate; whereas before medication, the daily output had been less than 0.25 gram.

4. There was little or no effect on the hemoglobin or red blood cell formation following injections of liver extract.

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# HEMOPHILIA. II. SOME PROPERTIES OF A SUBSTANCE OBTAINED FROM NORMAL HUMAN PLASMA EFFECTIVE IN ACCELERATING THE COAGULATION OF HEMOPHILIC BLOOD

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In previous studies (1) it was observed that citrated normal plasma rendered free of cellular elements by Berkefeld filtration, contained a substance which accelerated the clotting time of hemophilic blood. A preliminary report (2) indicated that the clot-promoting substance was associated with a saline soluble globulin fraction, as demonstrated by both *in vitro* and *in vivo* studies with hemophilic blood. The present report describes in detail the nature of the latter observations.

*Effect of temperature on the coagulation-accelerating substance contained in filtered<sup>1</sup> normal plasma.* Citrated normal plasma, which had been passed through a Berkefeld filter was diluted with 0.85 per cent NaCl solution to make one part of

saline to two parts of plasma. Dilution was used in order to bring out gradations in potency. Separate portions were then kept for a constant time, 16 hours, at 10°, 24°, 37°, and 46° C. Equivalent amounts were added by a standard technique<sup>2</sup> to hemophilic blood and the clotting times noted. A slight turbidity appeared in the portions kept at 37° and 46° C. but there was neither true precipitation nor bacterial growth. There was complete inactivation of the material when kept at 46° C. for sixteen hours. At the lower temperatures there was no essential change.

This study was then amplified to determine the effect of varying times of exposure to certain constant temperatures (Table I). Again filtered

<sup>1</sup> Hereafter in this paper the term "filter" will denote passage through the Berkefeld filter.

<sup>2</sup> This technique for testing the clot-promoting power of substances upon hemophilic blood was described in the first paper of this series (1).

TABLE I

*Effect on clotting time of hemophilic blood of adding filtered normal plasma which had been subjected to different temperatures*

	Clotting time Case I	Clotting time Case II
	minutes	minutes
2 cc. control hemophilic blood . . . . .	150	40
2 cc. control hemophilic blood plus 0.03 cc. plasma at 5° C. for 2 hours . . . . .	16	16
2 cc. control hemophilic blood plus 0.03 cc. plasma at 5° C. for 5 hours . . . . .	19	19
2 cc. control hemophilic blood plus 0.03 cc. plasma at 5° C. for 9 hours . . . . .	15	18
2 cc. control hemophilic blood plus 0.03 cc. plasma at 5° C. for 22 hours . . . . .	18	18
2 cc. control hemophilic blood plus 0.03 cc. plasma at 5° C. for 48 hours . . . . .	16	16
2 cc. control hemophilic blood . . . . .	120	40
2 cc. control hemophilic blood plus 0.03 cc. plasma at 37° C. for 2 hours . . . . .	16	16
2 cc. control hemophilic blood plus 0.03 cc. plasma at 37° C. for 5 hours . . . . .	21	19
2 cc. control hemophilic blood plus 0.03 cc. plasma at 37° C. for 9 hours . . . . .	15	17
2 cc. control hemophilic blood plus 0.03 cc. plasma at 37° C. for 22 hours . . . . .	23	23
2 cc. control hemophilic blood plus 0.03 cc. plasma at 37° C. for 48 hours . . . . .	23	23
2 cc. control hemophilic blood . . . . .	120	47
2 cc. control hemophilic blood plus 0.03 cc. plasma at 43° C. for 2 hours . . . . .	16	16
2 cc. control hemophilic blood plus 0.03 cc. plasma at 43° C. for 5 hours . . . . .	21	19
2 cc. control hemophilic blood plus 0.03 cc. plasma at 43° C. for 9 hours . . . . .	20	20
2 cc. control hemophilic blood plus 0.03 cc. plasma at 43° C. for 22 hours . . . . .	38	37
2 cc. control hemophilic blood plus 0.03 cc. plasma at 43° C. for 48 hours . . . . .	120	35
2 cc. control hemophilic blood plus 0.03 cc. supernatant fluid at 43° C. for 48 hours . . . . .	120	34
2 cc. control hemophilic blood plus 0.03 cc. precipitate in saline at 43° C. for 48 hours . . . . .	120	35

normal plasma was used. It was portioned by sterile technique into different tubes which were kept at 5°, 37° and 43° C., respectively, for varying intervals of time. Equivalent amounts of the plasmas so prepared were then tested as before for their coagulation-accelerating power on hemophilic blood. In 48 hours there was slight inactivation of the plasma kept at 37° C. and marked inactivation of that kept at 43° C. After 48 hours a precipitate formed in the plasma kept at 43° C. In this case, however, neither the whole plasma, the supernatant fluid, nor the precipitate dissolved in isotonic saline were found to be potent.

*The substance contained in filtered normal plasma which effectively reduces the clotting time of hemophilic blood is thermolabile.*

*Effect of dialysis partitions of filtered normal plasma on the clotting time of hemophilic blood.* As a first attempt at isolation of the active component of normal plasma a rough partition was tried by simple dialysis. Normal citrated plasma was passed through a Berkefeld filter and then was allowed to dialyze in cellophane jackets for eight days in jars of distilled water at room temperature. The water was changed twice daily. A white flocculent precipitate collected at the bottom of the jackets. The contents of the jackets were transferred to a test tube and the supernatant fluid removed from the precipitate by centrifugation. The precipitate remaining in the jackets together with that freed by centrifugation from the supernatant fluid was washed several times in distilled water and was then suspended in sufficient 0.85 per cent NaCl solution to give the same volume as that of the supernatant fluid. A sample of the parent filtered plasma was then diluted with saline so that its total volume equalled the combined volumes of the supernatant fluid and of the saline suspension of the precipitate. Comparison of the relative effectiveness of the three fluids was then made by adding 0.03 cc. of each to 2 cc. of hemophilic blood, and the clotting times determined.

The addition either of filtered plasma or of the saline suspension of the precipitate reduced sharply the clotting time of hemophilic blood (Table II), but the supernatant fluid was virtually ineffective. Chemical determinations of

TABLE II

*Effect on clotting time of hemophilic blood of adding dialysis partitions of filtered normal plasma*

	Clotting time	
	Case I	Case II
	minutes	minutes
2 cc. control hemophilic blood . . . .	120	43
2 cc. control hemophilic blood plus 0.03 cc. plasma . . . . .	22	13
2 cc. control hemophilic blood plus 0.03 cc. supernatant fluid . . . . .	100	45
2 cc. control hemophilic blood plus 0.03 cc. saline suspension of precipitate . . . . .	30	22

*Protein partitions of test substances*

	Total protein	Albumin	Globulin
	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.
Plasma (diluted) . . . . .	3.71	2.15	1.56
Supernatant fluid . . . . .	2.29	1.68	0.61
Saline suspension of precipitate . . . . .	1.47	0.323	1.15

the protein partitions of these fluids revealed that although complete separation was not accomplished, the saline suspension contained chiefly globulin, whereas the supernatant fluid contained chiefly albumin.

Both filtered plasma and a saline suspension of crude globulin remained effective when kept at 8° C. for 18 hours. The saline suspension of crude globulin manifested a thermolability similar to that of the parent plasma, which likewise was inactivated by exposure to 46° C. in a water bath for 18 hours.

*By dialysis of filtered normal plasma against distilled water a globulin-like precipitate is formed. When this precipitate is suspended in 0.85 per cent NaCl and tested in concentrations comparable to that of the supernatant fluid and to the parent plasma, the globulin portion retains most of the power of the plasma to shorten the clotting time of hemophilic blood. The effective material contained in the "globulin substance" is likewise thermolabile.*

*Preparation of globulin substance from plasma by dilution and acidification with CO<sub>2</sub>.* The observations recorded above indicated that a coagulation-accelerating substance was associated with

<sup>3</sup> This non-diffusible material, soluble in isotonic saline and associated with the globulin portion of plasma will be referred to hereafter as "globulin substance."

the globulin fraction of plasma. However, the separation by dialysis did not provide complete separation of globulin from albumin, and the method was awkward and impracticable. A more direct method was tried which was similar to that described by Addis (13) in the preparation of "fibrinogen solution," and later by Eagle (8) in the preparation of "prothrombin solution." This involved the well known effect of dilution and acidification of plasma. The globulin material so precipitated exhibited the characteristics of whole plasma in respect to the clotting of hemophilic blood.

Citrated plasma was obtained by the usual technique from fresh normal and hemophilic bloods respectively. Each sample was passed through a Berkefeld filter. To one part of each plasma were added nine parts of cold tap water, and the mixtures were then subjected to a stream of CO<sub>2</sub> for seven minutes, when a cloudy precipitate appeared. After centrifugation at 2,000 r.p.m. for 15 minutes, the clear supernatant fluid was discarded and the white gummy precipitate was suspended in 0.85 per cent NaCl and diluted with the saline solution to the original volume of the parent plasma. After 24 hours in the icebox at 8° C. a tough precipitate generally floated on top of the saline mixture (presumably fibrin). This precipitate was removed with a glass rod, and the remaining opalescent fluid was then tested by standard technique with hemophilic blood. The clotting time of 2 cc. of hemophilic blood was sharply reduced by the addition of 0.03 cc. of either normal plasma or its derived globulin substance, whereas the addition of equivalent amounts of hemophilic plasma or its derived globulin substance was relatively ineffective (Table III). Chemical analyses showed no appreciable differ-

ences in nitrogen content between the normal and hemophilic test substances.

Sometimes the normal globulin substance in 0.85 per cent NaCl lost its coagulation-accelerating power in several days, and yet other samples kept in the icebox retained potency after storage for several months. The cause of this discrepancy was not ascertained. It was not due to bacterial contamination. Exposure in a water bath to 56° C. for 5 minutes destroyed the coagulation-accelerating power of globulin substance so prepared.

*The effect of intravenous injections of normal globulin substance on the clotting time of hemophilic blood.* Globulin substance was prepared as in the previous studies, by dilution and acidification of plasma with CO<sub>2</sub>. The saline suspension was centrifuged at 2,000 r.p.m. for 10 minutes, and the opalescent supernatant fluid was passed through a Berkefeld filter. The saline solution was then administered intravenously by gravity infusion. Precaution was taken to flush the infusion apparatus with 0.85 per cent NaCl solution in order to avoid clot formation in the needle. Except for a sensation of slight fullness in the head, no untoward effects were experienced by the patients. The clotting times were materially reduced, but not so sharply as by transfusion with blood equivalent in amount to that from which the substance was derived (Table IV).

*The dilution of filtered normal plasma with water and the subsequent acidification with CO<sub>2</sub> resulted in the formation of a precipitate, which, when suspended in saline, was as effective as whole plasma in reducing the clotting time of hemophilic blood. The clot-promoting power of this normal globulin substance was effective both in vitro and in vivo. It was unstable and thermolabile. It*

TABLE III

*Effect on clotting time of hemophilic blood of adding "globulin substance" derived from filtered normal and hemophilic plasmas*

	Clotting time	
	Case I	Case II
	minutes	minutes
2 cc. control hemophilic blood . . . . .	180	72
2 cc. control hemophilic blood plus 0.03 cc. filtered normal plasma . . . . .	22	22
2 cc. control hemophilic blood plus 0.03 cc. normal globulin substance . . . . .	16	16
2 cc. control hemophilic blood plus 0.05 cc. normal globulin substance . . . . .	12	12
2 cc. control hemophilic blood plus 0.03 cc. filtered hemophilic plasma . . . . .	180	59
2 cc. control hemophilic blood plus 0.03 cc. hemophilic globulin substance . . . . .	180	52
2 cc. control hemophilic blood plus 0.05 cc. hemophilic globulin substance . . . . .	150	41



TABLE IV

*Effect on clotting time of hemophilic blood of intravenous injection of "globulin substance"*

	Clotting time	
	Case I*	Case II*
	minutes	minutes
Clotting time before injection.....	100	50
Clotting time 1 hour after injection....	30	32
Clotting time 2½ hours after injection..	49	28
Clotting time 4 hours after injection...	40	20
Clotting time 24 hours after injection..	85	35
Clotting time 96 hours after injection..		39

\* Case I received 135 cc. "globulin substance." Case II received 75 cc. "globulin substance."

*was either absent from or greatly diminished in globulin substance similarly prepared from hemophilic plasma.*

*Preparation of dried globulin substance. Comparison of CO<sub>2</sub> and acetic acid precipitation methods.* Because of the instability of a saline suspension of the globulin substance, the material was prepared in dried form. Two hundred cc. of fresh filtered normal plasma were divided into two equal portions. To 100 cc. were added 900 cc. of cold tap water, and the mixture subjected for one-half hour to a stream of CO<sub>2</sub> until pH 5.9 was reached. The cloudy fluid was centrifuged at 2,000 r.p.m. for one-half hour, the clear supernatant fluid decanted, and the precipitate, dried at room temperature in a vacuum desiccator, yielded 650 mgm. of grey-brown amorphous material.

The second portion of 100 cc. of plasma was similarly diluted with water and then acidified by adding 1 per cent acetic acid to pH 5.3. The cloudy fluid was likewise centrifuged at 2,000 r.p.m. for one-half hour, the supernatant fluid discarded, and the precipitate, dried at room temperature in a vacuum desiccator, yielded 713 mgm. of a similar grey-brown material.

Acetic acid precipitation was likewise used with fresh, filtered plasma from 2 cases of hemophilia, with yields of 452 and 670 mgm., respectively, per 100 cc. plasma.

In two instances, fresh normal blood was defibrinated by stirring with a glass rod and then centrifuged at 2,000 r.p.m. for one-half hour. The supernatant fluid was removed and passed through a Berkefeld filter. This defibrinated plasma was then subjected to dilution and acidification—the resultant precipitate dried, as above, with a yield of about 500 mgm. per 100 cc. plasma.

All materials were then pulverized and kept dry over calcium chloride in a desiccator. Samples of the dried powders so prepared were tested for their effect on the clotting time of hemophilic blood.

*Effect of adding dried globulin substance in vitro to hemophilic blood.* Ten milligrams of each dried material were emulsified and then taken up in 2 cc. of 0.85 per cent NaCl. The cloudy suspension was centrifuged at 1,500 r.p.m. for 15 minutes and 0.1 cc. of the clear supernatant fluid of each was tested with 2 cc. of hemophilic blood by standard technique.

Table V shows that the globulin substance from normal blood,<sup>4</sup> whether prepared from citrated plasma by CO<sub>2</sub> or acetic acid precipitation, was optimally effective in reducing the clotting time of hemophilic blood, whereas the hemophilic globulin substance, so prepared, was inert. The globulin substance from defibrinated normal blood, however, did not reduce the clotting time of hemophilic blood. In the process of defibrination the coagulation-accelerating material apparently was pre-

<sup>4</sup> The addition to hemophilic blood of dried "globulin substance" prepared from sheep, ox, rabbit, and monkey plasma showed a coagulation-accelerating effect similar to that obtained with the normal human derivative.

TABLE V

*Effect on clotting time of hemophilic blood of adding saline suspensions of dried "globulin substance" as prepared from normal plasma, from hemophilic plasma, and from defibrinated normal plasma*

	Clotting time	
	Case I	Case II
	minutes	minutes
2 cc. control hemophilic blood.....	100	42
2 cc. control hemophilic blood plus 0.1 cc. normal globulin by CO <sub>2</sub> method.....	9	8
2 cc. control hemophilic blood plus 0.1 cc. normal globulin by acetic acid method.....	9	8
2 cc. control hemophilic blood plus 0.1 cc. hemophilic globulin by acetic acid method.....	100	45
2 cc. control hemophilic blood plus 0.1 cc. defibrinated normal globulin by acetic acid method..	85	37

cipitated with the fibrin. That it was not identical with it, however, may be seen in the following studies.

*Physiological characteristics of the globulin substance prepared from normal and from hemophilic filtered plasmas.* Saline suspensions of the dried globulin substances as prepared in the preceding study, were tested by the method of Howell and Cekada (18) who used a calcium-fibrinogen system in their comparison of normal and hemophilic prothrombin. Calcium chloride was made up in 0.5 per cent aqueous solution. Fibrinogen solution was prepared from normal human blood according to the method of McLean (25). The addition successively of calcium chloride, of fibrinogen and of both calcium chloride and fibrinogen to a solution of the globulin substance was performed in standard test tubes. The tubes were shaken briefly and placed in a water bath at 37° C.

It was observed that the addition of calcium chloride alone to the globulin substance did not produce clotting. The addition of fibrinogen solution to the globulin substance produced no clot in six hours but did cause clotting in 24 hours. This suggested that the globulin substance, acting as prothrombin, was slowly converted into thrombin, which, as Mellanby (11) has shown, may take place in the presence of minute quantities of calcium or even in its apparent absence.

The addition of both calcium chloride and fibrinogen solutions to solutions of the hemophilic as well as of the normal globulin substance was followed by prompt clotting (Table VI).

According to the criteria of Howell and Cekada (18), these observations confirm their conclusions, namely, that quantitatively and physiologically both the normal and hemophilic prothrombins behave similarly. However, when solutions of these globulin substances were tested with hemophilic blood as in the preceding study, *only the normal globulin substance, unlike the hemophilic, caused a sharp reduction of clotting time.*

The clot formed by the combination of hemophilic globulin substance with calcium and fibrinogen reliquified in 12 hours, whereas the clot formed by combination with normal globulin substance reliquified only after 24 to 48 hours.

*In filtered normal plasma, after dilution with water and acidification with either CO<sub>2</sub> to pH 5.9 or with 1 per cent acetic acid to pH 5.3, there was formed a precipitate which, dried in vacuo, contained an active coagulation-promoting substance for hemophilic blood. Approximately 500 to 700 mgm. of the grey powder were obtained from 100 cc. of citrated normal plasma.*

*When the above procedure was applied to hemophilic plasma an approximately equivalent amount of dried material was produced, the saline suspension of which had little, if any, ability to hasten the clotting of hemophilic blood in the test tube.*

*Tested against a freshly prepared calcium fibrinogen system, both the normal and hemophilic precipitates were equally active as prothrombin. These observations seem to indicate that the difference between normal and hemophilic blood is due either to a qualitative difference of the globulin*

TABLE VI  
Clotting characteristics of dried "globulin substance" in relation to a calcium-fibrinogen system

A.	0.3 cc. normal globulin plus 0.1 cc. CaCl <sub>2</sub> .....	0 clot in 24 hours
	0.3 cc. hemophilic globulin plus 0.1 cc. CaCl <sub>2</sub> .....	0 clot in 24 hours
	0.3 cc. defibrinated normal globulin plus 0.1 cc. CaCl <sub>2</sub> .....	0 clot in 24 hours
B.	0.3 cc. normal globulin plus 0.4 cc. normal fibrinogen.....	0 clot in 6 hours (Clot in 24)
	0.3 cc. hemophilic globulin plus 0.4 cc. normal fibrinogen.....	0 clot in 6 hours (Clot in 24)
	0.3 cc. defibrinated normal globulin plus 0.4 cc. fibrinogen.....	Clot in 26 minutes
C.	0.3 cc. normal globulin plus 0.1 cc. CaCl <sub>2</sub> plus 0.4 cc. normal fibrinogen.....	Clot in 15 minutes *
	0.3 cc. hemophilic globulin plus 0.1 cc. CaCl <sub>2</sub> plus 0.4 cc. normal fibrinogen.....	Clot in 15 minutes *
	0.3 cc. defibrinated normal globulin plus 0.1 cc. CaCl <sub>2</sub> plus 0.4 cc. fibrinogen.....	Clot in 27 minutes
D.	0.5 cc. fibrinogen plus 0.3 cc. CaCl <sub>2</sub> .....	0 clot

\* The hemophilic globulin-calcium-fibrinogen clot reliquified in 12 hours, whereas the corresponding normal clot reliquified in 48 hours. The ready clotting of defibrinated plasma globulin in B as well as C indicates that thrombin was liberated by process of defibrination.

substance or to other substances associated with the globulin fraction of the plasma.

*The effect of reprecipitation of the globulin substance contained in normal plasma.* After precipitating globulin substance from filtered normal plasma by dilution and acidification to pH 5.3, the precipitate was taken up in 0.85 per cent NaCl to the volume of the parent plasma. This saline suspension of globulin was centrifuged at 2,500 r.p.m. for one-half hour in order to rid the mixture of particulate matter. The supernatant fluid was then diluted with 10 volumes of water, and a white flocculent precipitate was thrown down at pH 5.3. The precipitate was collected and dried in a vacuum desiccator. The yield of reprecipitated globulin substance was 430 mgm. per 100 cc. of plasma. When equivalent concentrations of the reprecipitated globulin substance were compared with the preparations obtained by a single precipitation, the reprecipitated globulin substance was found to be less active than the cruder material in its clot-promoting power on hemophilic blood. Refinement and concentration by this method, therefore, was unsuccessful.

*The chemical characteristics of globulin substance.* Globulin substance prepared from normal plasma gives the usual precipitation and color reactions of a protein, and the specific reactions of globulin. It is partially soluble in 0.85 per cent NaCl solution but wholly insoluble in distilled water at pH 6.5.

On analysis of samples of globulin substance from normal and from hemophilic blood, yields between 15 to 18 per cent of nitrogen were obtained, with the exception of one hemophilic globulin substance which contained 21 per cent nitrogen. The solubility in 0.85 per cent NaCl solution of

globulin substance prepared from plasma of different subjects is given in Table VII. The values are in terms of the percentage of nitrogen dissolved in isotonic saline.

Globulin substance from two normal individuals and from a patient with hemophilia whose blood clotted in between 40 and 60 minutes was found to be 50 to 60 per cent soluble in 0.85 per cent NaCl solution. Globulin substance from a case of hemophilia in which the clotting time was above 2 hours was 16 per cent soluble. Normal globulin substance reprecipitated from its saline solution showed a reduced solubility to 29 per cent in 0.85 per cent NaCl solution. Further studies are essential in order to determine whether these differences are due to intrinsic peculiarities of the globulin substances or whether they are due to unintentional variations in the technique of preparation.

*The coagulation-accelerating substance obtained from normal plasma is precipitated with a protein giving the characteristics of a saline soluble globulin.*

*Effect of variation in temperature on the coagulation-accelerating power of globulin substance obtained from filtered normal plasma.* One hundred mgm. of powdered globulin substance from normal blood was taken up in 20 cc. of 0.85 per cent NaCl solution. The insoluble matter was centrifuged down, and the clear supernatant fluid was pipetted by sterile technique into each of five tubes. Each tube was then kept at a different constant temperature. At intervals, 0.1 cc. of each fluid was removed from the tubes and tested in the usual manner against 2 cc. of hemophilic blood, and the clotting times noted with standard technique. All fluids remained clear except for those kept at 48° C., which acquired a flocculent precipitate after one-half hour.

It was observed (Table VIII, Figure 1) that at 48° C. there was loss of activity in 3 to 6 hours; at 43° C., in 6 to 10 hours; at 36° C. in 10 hours; at 25° C. in 22 hours; at 10° C. a slight loss in 48 hours. Like plasma, the coagulation-accelerating activity of globulin substance is thermolabile.

*Observations on the optimal hydrogen ion concentration for the precipitation of active globulin substance.* Since active globulin substance from normal plasma was obtained by precipitation either

TABLE VII  
Chemical analyses of "globulin substance"

Number	Source of globulin substance	Total nitrogen	Total nitrogen soluble in 0.85 per cent saline solution
		per cent	per cent
1	Hemophilic plasma	16.0	56.0
2	Normal plasma	15.0	61.5
3	Normal plasma	18.0	51.5
4	Hemophilic plasma	21.0	16.0
5	Number 2 reprecipitated	15.0	29.2

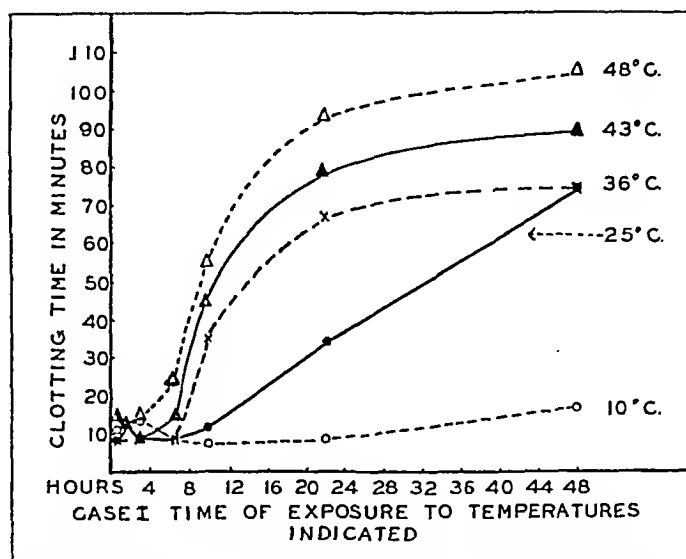


FIG. 1. EFFECT OF VARIATION IN TEMPERATURE ON THE ACTIVITY OF "GLOBULIN SUBSTANCE."

with  $\text{CO}_2$  at pH 5.9 or with 1 per cent acetic acid at pH 5.3 there apparently was a rather wide range of hydrogen ion concentrations within which the effective substance could be obtained. In order to determine the optimal conditions more precisely globulin substance was prepared from the

TABLE VIII

*Effect on clotting time of hemophilic blood of adding saline suspensions of dried "globulin substance" which had been subjected to constant temperatures for varying intervals of time \**

Case number	Time intervals	Clotting time					
		Controls	10° C.	25° C.	36° C.	43° C.	48° C.
	hours	minutes	minutes	minutes	minutes	minutes	minutes
I	$\frac{1}{2}$	126	11	9	9	14	14
	1	100	13	13	13	13	13
	3	120	14	9	9	9	15
	$6\frac{1}{2}$	125	9	9	9	15	24
	10	130	7	12	35	45	55
	22	120	9	34	67	79	92
	48	110	17	75	75	90	105
II	$\frac{1}{2}$						
	1						
	3	40	9	9	9	9	14
	$6\frac{1}{2}$	42	8	8	8	15	18
	10	40	7	8	20	23	35
	22	52	9	21	27	32	47
	48	40	13	34	35	35	40

\* In each tube were pipetted 0.1 cc. of the test solution and 2 cc. of hemophilic blood. In the control tubes were pipetted 0.1 cc. of saline plus 2 cc. of hemophilic blood. Clotting times were done by standard technique at 37° C.

same plasma at different hydrogen ion concentrations. This was done in the following manner: 400 cc. of filtered normal plasma was divided into 8 portions of 50 cc. each. These were diluted separately with 10 volumes of water. Varying amounts of 1 per cent acetic acid were added to each to obtain a series of pH ranges. The resultant mixtures were centrifuged at 2,000 r.p.m. for one-half hour, the precipitates removed, dried, and weighed. The respective weights of these precipitates are recorded in Table IX. Of each dried precipitate a 20 mgm. sample was suspended in 4 cc. of 0.85 per cent NaCl solution. The suspensions were centrifuged at 1,500 r.p.m. for 10

TABLE IX

*Yields of dried "globulin substance" as obtained by acidification of diluted normal plasma at different H ion concentrations \**

Plasma	Dilution in volumes	1 per cent acetic acid	Resultant pH	Yield after desiccation
cc.		cc.		mgm.
50	10	2	6.98	59
50	10	4	6.54	113
50	10	5	6.47	160
50	10	7	6.09	241
50	10	8	5.90	258
50	10	10.5	5.52	268
50	10	12	5.42	265
50	10	15	5.10	243

\* The yields recorded are merely approximate values.

TABLE X

Effect on clotting time of hemophilic blood of saline suspensions of dried "globulin substance" obtained at different H ion concentrations from normal plasma

	Clotting time				
	Case I	Case II	Case III	Case IV	Case V
	minutes	minutes	minutes	minutes	minutes
Control hemophilic blood.....	140	120	100	51	37
Control hemophilic blood plus 0.1 cc. at pH 5.1.....	62	21	15	12	10
Control hemophilic blood plus 0.1 cc. at pH 5.42.....	54	20	14	10	10
Control hemophilic blood plus 0.1 cc. at pH 5.6.....	23	13	14	10	7
Control hemophilic blood plus 0.1 cc. at pH 5.8.....	20	8	7	6	6
Control hemophilic blood plus 0.1 cc. at pH 6.09.....	18	8	7	5	3
Control hemophilic blood plus 0.1 cc. at pH 6.47.....	17	8	7	6	3
Control hemophilic blood plus 0.1 cc. at pH 6.54.....	22	13	7	6	4
Control hemophilic blood plus 0.1 cc. at pH 6.98.....	52	30	7	12	11

minutes and the clear, supernatant fluid tested by standard technique against hemophilic blood.

It was noted that the substance obtained by precipitation at pH 5.9 to pH 6.4 was the most effective in reducing the clotting time of hemophilic blood (Table X; Figure 2). While only one set of observations is recorded, the procedure has been repeated with practically identical results.

The effect of dilution on the activity of dried globulin substance obtained from normal filtered plasma. Fifty mgm. of dried globulin substance precipitated at the optimal point of pH 6.09 was taken up in 5 cc. of 0.85 per cent NaCl solution. After thorough mixture, this was centrifuged at 2,000 r.p.m. for 15 minutes, and the clear, super-

natant fluid removed. Serial dilutions of the supernatant fluid were made with 0.85 per cent NaCl up to 1:256 dilution. Of each dilution 0.1 cc. was added to 2 cc. of hemophilic blood by standard technique, and the clotting times noted. In Table XI and Figure 3, the effect of dilution upon the coagulation-accelerating activity of the globulin substance from normal plasma is illustrated.

By increased concentration of globulin substance

TABLE XI

Effect of dilution on the coagulation-accelerating power of "globulin substance" prepared from normal plasma \*

Dilution with saline	Clotting time				
	Case I	Case II	Case III	Case IV	Case V
	minutes	minutes	minutes	minutes	minutes
0 dilution.....	8	5	4	5	5
1 : 2 dilution.....	26	7	6	12	8
1 : 4 dilution.....	50	9	17	15	12
1 : 8 dilution.....	64	12	17	19	15
1 : 16 dilution.....	88			26	27
1 : 32 dilution.....	105	28	23	42	40
1 : 64 dilution.....		40	43	44	42
1 : 128 dilution....		57	73	49	46
1 : 256 dilution....		62	70	48	47
Control hemophilic blood.....	100	82	73	58	49

\* 50 mgm. of dried "globulin substance" was taken up in 5 cc. of 0.85 per cent NaCl. After centrifuging the suspension at 1,500 r.p.m. for 10 minutes, serial dilutions of the supernatant fluid were made as indicated and 0.1 cc. of each dilution added by standard technique to 2.0 cc. of hemophilic blood.

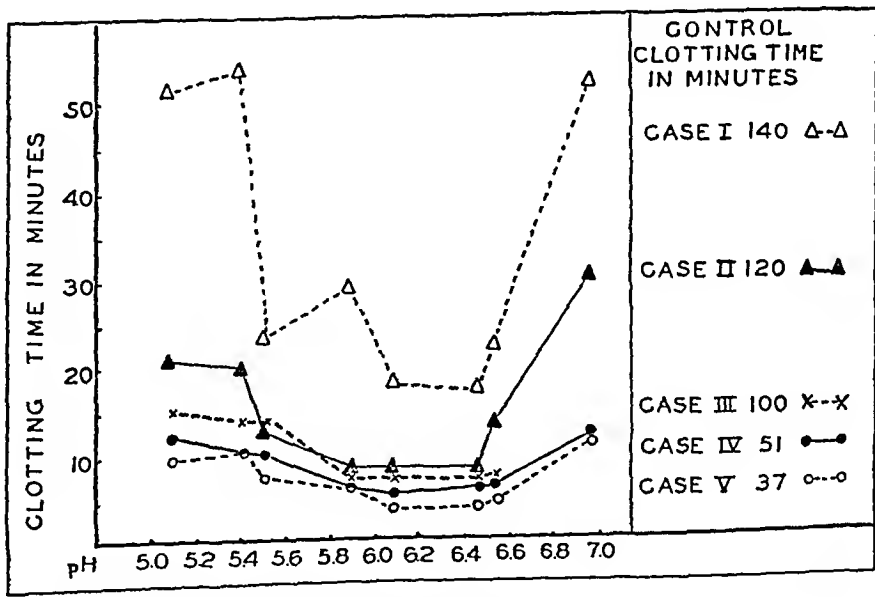


FIG. 2. EFFECT OF VARIATION IN pH OF DILUTED PLASMA ON THE ACTIVITY OF PRECIPITATED "GLOBULIN SUBSTANCE."

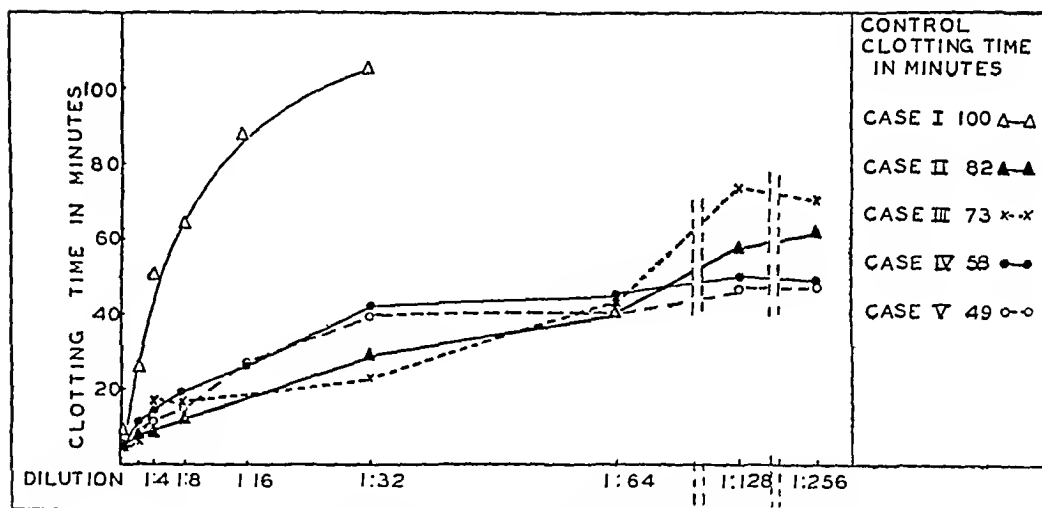


FIG. 3. EFFECT OF DILUTION ON ACTIVITY OF "GLOBULIN SUBSTANCE."

prepared from hemophilic plasma a coagulation-accelerating substance of minimal activity was obtained. Solutions of hemophilic globulin substance were prepared in such concentration that 0.1 cc. contained 2 mgm. The addition of 0.1 cc. of such hemophilic globulin substance to 2 cc. of hemophilic blood reduced the clotting time with about the same effectiveness as a solution of globulin substance prepared from normal plasma, in which 0.1 cc. contained 0.0625 mgm. In coagulation-accelerating power the hemophilic globulin substance was approximately equal to 1:32 dilution of the normal derivative. This indicated that in the hemophilic the substance is greatly diminished in amount but not absent.

*The intravenous injection of a saline solution of dried normal globulin substance and its effect on the clotting time of hemophilic blood.* One gram of dried normal globulin substance was taken up in 200 cc. of 0.85 per cent NaCl solution. After centrifugation at 2,000 r.p.m. for 15 minutes, approximately 0.5 gram was recovered, indicating that about 50 per cent was soluble. The supernatant fluid was passed through a Berkefeld filter (Grade V) and administered by gravity infusion as described previously. The procedure was performed in 3 cases. When injected in about 10 minutes, the patients experienced a sensation of fullness and warmth. In two instances the injection was followed in 1 hour by moderate chill and headache for 10 minutes. In the third in-

stance, when the material was injected during a period of 30 minutes, no untoward reaction occurred.

There followed in each case a definite reduction of clotting time (Table XII and Figure 4) which was sustained for 10 hours in 2 cases and for 24 hours in the third. The response was slower than that occurring after blood transfusion (1). The material used in Case II was freshly prepared

TABLE XII

*Effect on clotting time of hemophilic blood of the intravenous injection of saline solution of dried "globulin substance" prepared from normal plasma*

Case number	Clotting time
	minutes
I	Clotting time before injection . . . . . 135
	Clotting time 40 minutes after injection . . . 60
	Clotting time 70 minutes after injection . . . 70
	Clotting time 3 hours after injection . . . . 48
	Clotting time 5 hours after injection . . . . 45
	Clotting time 9½ hours after injection . . . . 70
	Clotting time 24 hours after injection . . . . 122
II	Clotting time before injection . . . . . 165
	Clotting time 45 minutes after injection . . . 23
	Clotting time 2 hours after injection . . . . 25
	Clotting time 5 hours after injection . . . . 24
	Clotting time 10½ hours after injection . . . . 30
	Clotting time 24 hours after injection . . . . 58
III	Clotting time 48 hours after injection . . . . 150
	Clotting time before injection . . . . . 150
	Clotting time 75 minutes after injection . . . 35
	Clotting time 3 hours after injection . . . . 30
	Clotting time 5 hours after injection . . . . 41
	Clotting time 24 hours after injection . . . . 83

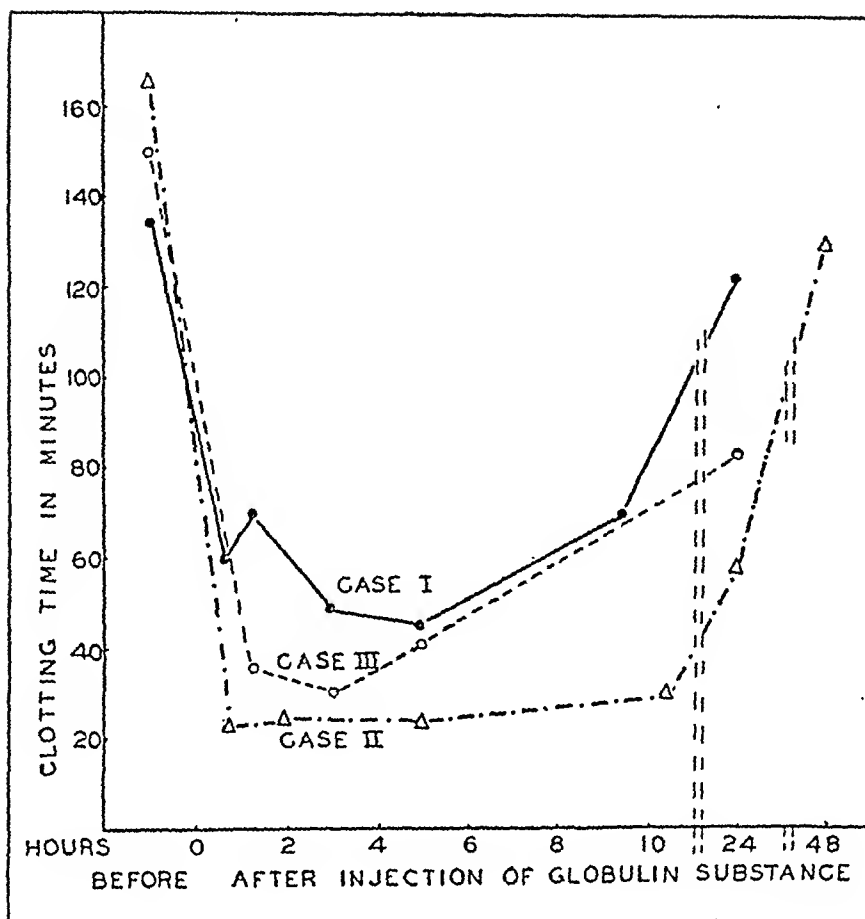


FIG. 4. EFFECT OF INTRAVENOUS INJECTION OF "GLOBULIN SUBSTANCE" ON CLOTTING TIME OF HEMOPHILIC BLOOD.

at pH 6.09 and was shown by *in vitro* tests to reduce the clotting time from 165 minutes to 6 minutes; whereas that which was used in Cases I and III was made from old, pooled plasma which by *in vitro* tests reduced the clotting time from 150 to 30 minutes.

A control study was performed with globulin substance similarly prepared from 550 cc. of hemophilic blood, which yielded 1.2 grams of dry powder. The hemophilic globulin substance was obtained from a patient whose blood had a clotting time of 50 minutes, and it was injected into another patient whose blood clotted in 150 minutes. One hour after injection the recipient's clotting time was 85 minutes. Subsequent tests, however, made 3 hours, 5 hours, 10 hours, and 24 hours after the injection showed clotting times between 120 and 155 minutes. *In vitro* tests showed a comparable minimal reduction in clotting time.

These results may indicate that the material obtained from the mild hemophilic patient provided a small amount of coagulation accelerating sub-

stance. The results confirm previous observations which demonstrated the specificity of the reduction in hemophilic clotting time following the injection of normal globulin substance.

*Dried normal globulin substance, when taken up in 0.85 per cent NaCl solution, exhibits the coagulation-accelerating properties of the freshly prepared material. The optimal zone for precipitation of this substance lies in the pH range of 5.9 to 6.4. It manifests a characteristic pattern of reaction to dilution and to changes of temperature. Intravenous injection of a saline solution of dried normal globulin substance in 3 cases of hemophilia was followed by definite reduction of the clotting time of the blood.*

#### COMMENT

Discussion of the relationship of globulin substance to other factors in blood coagulation necessarily involves a consideration of prothrombin. Whereas some authors (3, 4, 5, 6), identify the source of prothrombin, or a comparable sub-

stance, with platelets as well as plasma, others (7, 8), imply that it resides chiefly in the non-cellular plasma. Certain authors (9), indeed deny its existence. Its chemical nature and mode of activity are not clearly established. It is known only by its capacity to form thrombin. At present, therefore, it is better regarded as a physiological complex than a single, chemical substance.

There have been several methods described for its preparation. Mellanby (10) obtained it from bird plasma by precipitation, at a reaction of pH 5.3, of the globulin complex from diluted plasma. He later modified this by additional treatment with dilute calcium bicarbonate solution (11). Bordet and Delange (12) applied the principle of adsorption with tricalcium phosphate for the preparation from plasma of proserozyme (prothrombin). Addis (13) and later Eagle (8) precipitated it by dilution of plasma with water and acidification with  $\text{CO}_2$ . Howell (4) obtained it by acetone precipitation of plasma. Because of the varied methods for preparation and the failure to obtain a pure substance there is not complete agreement over the properties of prothrombin. In general, it is thought to be a protein, of globulin nature, nondialyzable, insoluble in distilled water but soluble in weak alkali. Its reaction to changes of temperature have been variously described.

A number of communications have attributed the clotting abnormality of hemophilia to altered prothrombin. Addis (13), Christie and his co-workers (6), Feissly and Fried (14) believed the delayed clotting resulted from a qualitative change in this substance. Klinger (15), Hurwitz and Lucas (16), and Howell (17) said there was a quantitative defect, but the latter reversed his opinion later (18). Govaerts and Gratia (19) concluded that a dual mechanism was involved, namely, that a plasma factor in normal blood activated the abnormally stable hemophilic platelets. The plasma factor was neither proserozyme (prothrombin) nor cytozyme (thrombokinase).

The present studies, we believe, clarify in a measure this confusion. If a scheme for blood clotting is accepted that involves only prothrombin, calcium, and fibrinogen, both normal and hemophilic prothrombins function similarly.

However, the addition of normal prothrombin accelerates the clotting of hemophilic blood, whereas the addition of hemophilic prothrombin does not. Hence, regardless of its behavior in a calcium fibrinogen system, there must be a specific alteration in the hemophilic "prothrombin complex."

The clotting substance described here is not dialyzable, but it does pass through a Berkefeld filter. Its range of optimal precipitation from plasma lies between pH 5.9 and 6.4. It is thermolabile, insoluble in water at pH 6.5, but soluble in isotonic saline. The substance so obtained either gives reactions of a protein with the characteristics of globulin or is associated with such a material. However, hemophilic plasma yields a similar material which has only minimal coagulation-accelerating activity. *Therefore, one may conclude at this juncture only that the clotting substance is precipitated with globulin*, but there is no proof that it is globulin itself.

Reiner and Reiner (20) have shown that there are at least two serum globulins with precipitation points at pH 5.1 and 6.8 respectively. It is possible that the hemophilic abnormality is associated with only one of several globulins. It is also possible that the active principle in globulin substance is an enzyme. Its possible protein nature, its potency in small amount, its pattern of reaction to changes in temperature and to dilution, its specific range of precipitation from plasma invite such an hypothesis.

Heretofore, the clotting abnormality of hemophilia has been studied *in vitro*. Bendien and Creveld (21, 22) implied that *in vivo* studies with a substance they isolated were promising, but no data were given. The fact that normal globulin substance reduces the clotting time *in vivo*, we believe changes the complexion of the disease from an abnormality that was immutably fixed to one that is amenable to change. Likewise the preparation of a relatively stable dry substance makes practical the further study of its properties.

Since the exact nature of globulin substance is not definable, comparison with other substances that are said to be active coagulation-accelerators in hemophilia would be untimely. Recent studies with vitamin K (23) may provide analogies. The placental coagulant described by Eley and his coworkers (24) may contain globulin sub-



stance since placenta is rich in blood. However, in several respects the two substances differ. More closely similar appears to be that described by Bendien and his coworkers (21) in Holland.

Obviously much remains to be learned: a more exact definition of "globulin substance"; its metabolism; its relation, if any, to enzymic processes; and ultimately its relation, if any, to the peculiar sex linkage that characterizes the disease.

#### SUMMARY AND CONCLUSIONS

1. Upon dilution and acidification of filtered normal plasma there is formed a globulin precipitate, which, either fresh or dried *in vacuo*, contains a clot-promoting substance for hemophilic blood. This substance is effective both *in vitro* and *in vivo*.

2. When the above procedure is applied to hemophilic plasma an approximately equivalent amount of precipitate is formed, the saline suspension of which has very little ability to hasten the clotting of hemophilic blood.

3. Tested with a calcium-fibrinogen system both normal and hemophilic precipitates are equally active as "prothrombin."

4. In respect to clotting activity "globulin substance" so prepared from normal plasma is thermostable, insoluble in water at pH 6.5, but soluble in isotonic saline. It is not ultrafilterable, but it does pass through a Berkefeld filter. Its range of optimal precipitation from plasma is between pH 5.9 and 6.4.

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# THE ARTIFICIAL INDUCTION OF SUBCUTANEOUS NODULES IN PATIENTS WITH RHEUMATIC FEVER<sup>1</sup>

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Although subcutaneous nodules in subjects with rheumatic fever were described prior to the report of Barlow and Warner (1), these workers presented the first series of observations on 27 patients and described the clinical characteristics of nodules. Like Cheadle (2), these observers believed subcutaneous nodules to be diagnostic of rheumatic fever. The early literature has been adequately summarized by Fitcher (3), and McEwen (4) has recently reviewed the more recent reports.

Subcutaneous nodules are usually multiple and may appear in crops over the bony prominences and sometimes on tendons. They are painless, non-tender, vary considerably in both size and consistency, and may be present from a few days to several months. Nodules are most apt to occur in patients with the more severe forms of rheumatic heart disease and many observers consider them to indicate a grave prognosis.

Drewitt (5) first hypothesized that trauma was involved in the production of subcutaneous nodules because of their common occurrence over bony prominences. In confirming the many observations on the location of subcutaneous nodules, we were impressed with the possibility that trauma may be a factor in their appearance. Interest in such a probability resulted in an attempt to traumatize the elbows of rheumatic fever subjects by several methods to see if subcutaneous nodules could be artificially induced. The results of these attempts to reproduce subcutaneous nodules form the basis of the present report.

## CLINICAL MATERIAL

The present report is based on studies upon 116 patients who are divided into the following groups:

(1) Sixty patients had rheumatic fever, exclusive of those with chorea. Twenty had clin-

ical manifestations of the disease, twenty-six were convalescing from clinical rheumatic fever but at the time of observation had only laboratory evidence of infection (i.e., an elevated sedimentation rate, leukocytosis, or prolongation of auriculo-ventricular conduction time by electrocardiogram), and fourteen were convalescing from rheumatic fever but at the time studied exhibited no clinical or laboratory evidence of active disease.

(2) Twenty-two patients had active or subsiding chorea as their presenting sign. Six of these had rheumatic heart disease, one had congenital heart disease, and in fifteen there was no clinical evidence of cardiac involvement. Six had chorea as their only manifestation of rheumatic fever, and sixteen had other symptoms of rheumatic fever in the past or at the time of the test. At the time of the study the degree of chorea varied from minimal to moderate.

(3) Thirty-four subjects with diseases other than rheumatic fever were used as controls. Thirty of these had bone tuberculosis of varying severity, one had hysteria, one habit spasm, one was convalescing from pneumonia, and one had subacute bacterial endocarditis.

The ages of the subjects composing these three groups were comparable, averaging 10 years and ranging between 4 and 16 years.

## METHODS

The chief method of approach in our attempt to induce the formation of nodules, and that used on each of the 116 patients of this series, was as follows.

The region of the olecranon process was infiltrated with 1 cc. of 1 per cent novocaine. From the antecubital vein of the opposite arm 2 to 3 cc. of blood was removed and immediately injected into the subcutaneous and deep tissues of the anesthetized area. During the next ten days, frictional pressure was applied by having the patient rub the injected elbow on the bedclothes for

<sup>1</sup> The expenses of this study have been defrayed by a grant from the Commonwealth Fund.

several minutes (or until the skin became warm), six times a day.

In addition to this primary method, the following procedures were used.

(1) Five subjects had one elbow tested in the above mentioned manner, and the other elbow injected only with novocaine before frictional pressure was applied.

(2) Fifteen patients tested by the primary method had only frictional pressure to the other elbow.

(3) Nine patients were injected by the basic method over both elbows, but frictional pressure was applied to only one elbow.

(4) Finally, twenty patients had normal saline substituted for blood in injecting the other elbow.

RESULTS

A hematoma developed over the elbow in those cases in which blood was injected. In some individuals the diffuse subcutaneous thickening subsided, and the tissues regained their normal texture by the end of one to two weeks. In others, as the hematoma subsided there appeared, usually by the end of the first or during the second week, a discrete, moveable, subcutaneous nodule-like structure. In the beginning, this was poorly defined and somewhat soft, but usually within another week or more it became definitely circumscribed and firm. The size varied from about 2 to 10 mm. in diameter. Clinically, these induced nodules could not be distinguished from the subcutaneous nodules which appear spontaneously in patients with rheumatic fever.

Table I summarizes the results of these experiments involving the subcutaneous injection of blood in 116 individuals.

Of twenty patients with clinical rheumatic fever, eighteen (90 per cent) developed nodules at the site of injection, and two-thirds were of moderate size or larger.

Of twenty-six patients with only laboratory evidence of active rheumatic fever, thirteen (50 per cent) exhibited a nodule response. Five of the nodules were of moderate to large size, and eight were small but definite.

Of fourteen patients without clinical or laboratory evidence of active infection, only two (14

TABLE I  
*Distribution and size of induced nodules*

Group	Number of cases tested	Successful nodule reaction		No nodule reaction		Size of induced nodules	
		Number	Per cent	Number	Per cent	Small	Moderate or larger
Clinical rheumatic fever.....	20	18	90	2	10	6	12
Rheumatic fever—abnormal laboratory tests only.....	26	13	50	13	50	8	5
Inactive rheumatic fever.....	14	2	14	12	86	2	0
Chorea.....	22	3	14	19	86	3	0
Non-rheumatic (controls)....	34	1	3	33	97	1	0
Total cases.....	116	37	32	79	68	20	17

per cent) developed nodules, and in both instances they were of small size.

The twenty-two patients with chorea as their presenting symptom have been classified separately. Only three subjects (14 per cent) developed nodules, and in each instance the nodule was of small size. One had laboratory evidence of active rheumatic fever. The second was convalescent from frank rheumatic fever, but at the time of injection had no clinical or laboratory evidence of activity. The third had chorea as the only known manifestation of rheumatic fever.

Of the thirty-four control patients, one (3 per cent) developed a definite nodule. This patient had low-grade fever, which may be explained by his active bone tuberculosis. However, in the past, he had spontaneous nosebleeds, and on examination there was a question as to the presence of an early aortic diastolic murmur. No definite diagnosis of rheumatic fever could be made.

These results apply only to those instances in which blood was injected subcutaneously into an anesthetized area overlying the olecranon process and the elbow then rubbed so as to produce frictional pressure. The results of the four additional procedures are also of interest and follow.

Four of five rheumatic patients who had blood injected by the basic method developed nodules after the maneuver, while the injection of novocaine alone into the other elbow and subsequent frictional pressure failed to cause a nodule response in any instance.

None of the fifteen patients who had only frictional pressure applied to one elbow developed nodules, but six of these same patients exhibited

a nodule response on the elbow where blood was injected.

Nine patients had blood injected subcutaneously into both elbows with frictional pressure to one elbow only. Five developed nodules bilaterally, while four had no reaction over either elbow.

Twenty individuals were injected with blood in one elbow and saline in the other. Six developed nodules at the sites of blood injection, and in four of these, nodules resulted also over the elbow, injected with saline.

#### DISCUSSION

It has been demonstrated that the injection of blood subcutaneously over the anesthetized olecranon process with subsequent frictional pressure resulted in the appearance of a nodule in the injected area in 37 (45 per cent) of 82 subjects with rheumatic fever and chorea. In no instance was friction alone a sufficient stimulus to induce the formation of a nodule. Nodules did appear over the areas where blood was injected, and in which there was no subsequent frictional pressure. Nodules did not appear as the result of infiltration with small amounts of novocaine.

From the evidence available it seems possible that tissue injury, if sufficient, may be of primary importance, although blood as a stimulant in itself cannot be ruled out. Of six individuals in whom the injection of blood induced nodules four had nodules appear also over the elbows in which saline was substituted for blood. The injection of 2 to 3 cc. of saline subcutaneously is necessarily accompanied by bleeding. At this time it would be mere speculation to make definite implications with regard to the actual process whereby these nodules appear.

In the rheumatic fever subjects injected, these induced nodules were definitely related to the activity of the rheumatic fever process. The appearance of nodules in 90 per cent of those patients with clinically active rheumatic fever, and in 50 per cent of those with only laboratory evidence of active rheumatic fever (67 per cent of the combined groups), was in striking contrast to their appearance in only 14 per cent of rheumatic fever subjects without evidence of active rheumatic fever, and in 14 per cent of those sub-

jects with chorea. From the above, it was evident that these induced nodules occurred more frequently in the patient with active rheumatic fever, as is the case with spontaneous nodules.

The size of the induced nodules also varied to some extent with the degree of activity of rheumatic fever. Thus, in the group with clinical rheumatic fever, two-thirds of the nodules were of moderate size or larger, while slightly less than one-half were of this size in the group with only laboratory evidence of active rheumatic fever. In the subjects with inactive rheumatic fever and chorea, all induced nodules were small.

Similarly, the duration of the induced nodules varied from a few weeks to several months, lasting the relatively longer periods in those individuals who had persistent infection. Their clinical course was hence comparable to the nodules observed to occur spontaneously in subjects with rheumatic fever.

These facts suggest a clinical similarity to spontaneous nodules.

A nodule appeared in only one (3 per cent) of the 34 control subjects, and the possibility has been mentioned that this child may be suffering from rheumatic fever. More adequate control studies are necessary before definite conclusions may be reached concerning the specificity and significance of these induced nodules. Therefore, it is not possible at this time to suggest that the induction of such nodules could be used as a diagnostic test of the presence or absence of rheumatic fever.

The histopathology of these induced nodules, and their comparison with spontaneous nodules will form the basis of the succeeding report (6).

#### SUMMARY

(1) The injection of the patients' own blood into the subcutaneous tissues of subjects with rheumatic fever frequently results in the appearance of subcutaneous nodules in the area injected. They are clinically indistinguishable from nodules occurring spontaneously.

(2) The same procedure carried out in thirty-four presumably non-rheumatic individuals resulted in nodule formation in a single instance.

The authors wish to express their appreciation to Mrs. Nellie S. Smith and to Dr. Gerald N. Hoeffel of the New

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# THE PATHOLOGY OF SPONTANEOUS AND INDUCED SUBCUTANEOUS NODULES IN RHEUMATIC FEVER<sup>1</sup>

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The purpose of this report is, first, to present a composite histological picture of subcutaneous nodules of rheumatic fever as obtained from a review of the literature; second, to attempt a pathological description on the basis of their known clinical age; and third, to describe the structure of a number of artificially induced nodules and compare them with spontaneously occurring nodules in rheumatic fever subjects. The production of these induced nodules and their clinical similarity to spontaneous nodules is the subject of the previous report (1).

Since the descriptions of subcutaneous rheumatic nodules found in the literature are very conflicting, it is desirable to summarize the available information to determine if the wide variations of structure are consistent with the findings which we propose to present. In the review of these reports, it is surprising that the descriptions are in most instances limited to single nodules removed at death. Only the more adequate descriptions will be quoted.

## LITERATURE

Barlow and Warner (2) in 1881 described the subcutaneous nodule as "consisting of small masses of loose fibrous bundles, sometimes very vascular." Hirschsprung (3) reported that nodules consisted of "connective tissue rich in cells similar to granulation tissue with necrobiosis." Cavafy (4) described two nodules, "a small one consisted of young actively growing fibrous tissue cells with much intercellular tissue," and a "larger one had much looser structure though consisting undoubtedly of fibrous tissue. Strands of fibers were separated widely by edema and cellular infiltration. . . . Both nodules were very vascular, and the small arteries were enormously thickened in the inner coat so as to obliterate the lumen of some of the vessels. Many of them showed only proliferation of the endothelium." Later he (5) described in addition "a peculiar reticular tissue with strikingly rectangular meshes. . . . in some parts this peculiar substance was fibrinous." Money (6) and Parker (7) very briefly described several nodules but added no further

information. Middleton (8) considered the nodule to be "made up of fibrous tissue in various stages of development, . . . at its periphery the arteries seem to be abnormally numerous and in many instances the coats are greatly thickened by infiltration, the intima being particularly affected. Collections of cells frequently extend to a considerable distance from the vessels." Mitchell (9) reported a nodule described by Osler as "a dense fibrous stroma with cells chiefly ovoid, the ends prolonged into fibrils. There was no arrangement of round celled elements as in granulation tissue." Nepveu (10) removed a nodule 36 hours after its appearance and reported "two small foci of granulated necrotic material with very faintly staining cells and necrotic fibrils of connective tissue. There is a zone of infiltration of leukocytes around the areas with transition of cells into necrosis." Fletcher (11) next described a nodule thus, "portions are composed largely of cellular elements, which under high power are seen to consist of small round cells, fibroblasts, and polymorphonuclear leucocytes. In these situations blood vessels are quite numerous so that to a certain extent there is a resemblance to granulation tissue. Several giant cells were present in younger portions of the nodule. . . . Some sections show a very interesting feature in the occurrence of a definite hyaline degeneration of the fibrous tissue in certain situations. . . . Portions of the fibrous tissue which have undergone hyaline change show quite marked cellular infiltration. . . . The vascularity of the nodule is a striking feature. The blood vessels are most numerous at the periphery of the nodule, the center being comparatively free excepting in the areas of cellular infiltration where minute vessels are present. Some larger vessels show an infiltration of small round cells into their walls, these in some instances extending some distance into the surrounding tissue. . . ." Poynton and Still (12) described a nodule removed three weeks after its appearance. "In the center of the nodule there is a homogeneous material arranged in layers and free of cellular elements. . . . There is fibrin, in the interstices of which there was originally fluid. . . . Toward the periphery many cellular elements can be seen encroaching on this fibrinous center. . . . Still further to the periphery fibrous tissue is apparent, some swollen and hyaline and in places there are distended and distorted vessels."

Wick (13) reported a correct objective description of a nodule, but misinterpreted the constituents. "The nodule consists of connective tissue moderately rich in cells and vessels, and inclosing several foci of various size and peculiar structure. The periphery of these foci consists of closely arranged cells of round, elongated, notched, or

<sup>1</sup> The expenses of this study have been defrayed by a grant from the Commonwealth Fund.

irregular shape. Most of these cells have a comparatively large nucleus and may be called epithelioid cells." Coombs (14) briefly described a nodule and remarked on the presence of multinuclear cells. Frank (15) reported on the histopathology of three nodules removed at death. ". . . a hyaline homogeneous substance is found (in the center) which stains intensively with eosin and contains a large amount of fibrin, as is seen with special staining. This hyaline substance penetrates between the fibrils of the connective tissue, the nuclei of which are only partially and poorly stained. . . . The periphery exhibits marked growth of connective tissue, which consists of young, fusiform, or epithelioid cells, and of fibrils of connective tissue with numerous thin, elongated cells. Moderately numerous leukocytes are found in this young growing tissue." Tilp (16) described a number of nodules. "The smaller nodules consist of relatively large, round or pear-shaped endothelioid cells which are often arranged around capillaries or precapillaries with small round cells disseminated between them. The periphery of the nodules consists of radially arranged, predominantly fusiform cells with rod or comma-shaped nuclei. . . . Bundles of fibrous connective tissue invade the marginal areas. The larger nodules all exhibit a central area consisting of filamentous masses, and of serous fluid. There are no nuclei in these central areas, but at the margins larger cells are seen in groups, particularly in the neighborhood of small blood vessels. . . ."

In 1924, Swift (17) described the structure of subcutaneous nodules as, "In close apposition to areas of cellular proliferation there is tissue destruction varying in size from submiliary areas to long strands of hyaline necrosis affecting connective tissue fibers; combined with necrosis are deposits of fibrin. Surrounding these foci are numerous cells similar in appearance and staining reaction to the type of cells found in Aschoff bodies; multinuclear giant cells are also present." He further remarks on the vascularity and vessel damage noted by previous writers and states, "A participation of fibroblasts arising from the connective tissue is easy to demonstrate. A few polymorphonuclear leukocytes and lymphocytes invade the diseased tissue and foci of edema are demonstrable. . . . The larger nodules are composed of a conglomeration of submiliary nodules." Coates (18) described two nodules and states, "Some of arterioles show punctate proliferation of endothelial lining. Perivascular mononuclear proliferation is frequent. The vessels present intimal and sub-intimal cellular proliferation and necrosis and are in an early stage of endarteritis." MacCallum (19) gives one of the best descriptions of the cellular constituents of nodules to be found in the literature. ". . . whenever a distinct line of demarkation is visible between this (central necrotic tissue) and living tissue, one may find a palisade-like arrangement of large cells with an irregular cell body and a large deeply stained vesicular nucleus or sometimes several nuclei. There is no distinct granulation of the cytoplasm of these cells, but their protoplasmic processes extend among the neighboring cells. Outside this layer,

which may be broad, there appears a highly vascular mass of branching and anastomosing fibroblast-like cells which form a coherent tissue in which wander a few leukocytes frequently with eosinophil granulations and rather more mononuclear wandering cells. It is difficult to feel sure of the nature of the large cells that form the immediate mantle about the necrotic material. . . . The nucleus is large and vesicular, usually with a very distinct nucleolus and many irregularly arranged chromatin particles. Some have several nuclei each with a distinct nucleolus. . . . They are paler than nearby endothelial cells, and they seem to form a tissue—at least they are the only cells in a tissue that shows intercellular fibrils. . . . The trend of their form is that of the tissue and they are distinctly and finely branched and attached to their neighbors by these branches. . . . (they) seem slightly different from fibroblasts and rouse the question of their possible closer relation to such phagocytic mononuclear wandering cells or clasmatocytes as are so generally concerned when necrotic tissue is present."

Coates and Coombs (20), Merritt (21), and Clawson (22) reported on the structure of nodules but contributed no further information on their histopathology. Dawson and Boots (23) in a paper on subcutaneous nodules in rheumatoid arthritis describe the structure of nodules which by inference applies as well to those of rheumatic fever. This description is as follows: "1. An area of central necrosis due in its earliest stages to a gelatinous swelling and disintegration of collagen bundles. Depending on the age of the nodule and the severity of the process there is a variable amount of fibrin deposition and inflammatory cell infiltration. The vessels in the nodule itself rarely show significant changes, but the arteries and capillaries in surrounding tissue show: (a) subendothelial deposition of fibrin, hyperplasia of subendothelial cells, narrowing and even occlusion of the lumen, and in some instances canalization of the hyperplastic intima; (b) splitting of the elastica and occasional formation of a new elastic coat; (c) perivascular cell infiltration by large mononuclear and small round cells. 2. A surrounding zone of peculiar and characteristically arranged large mononuclear cells. These cells are for the most part disposed in radial fashion and are largely responsible for the characteristic appearance of the whole lesion. 3. An inclosing zone of dense and relatively avascular fibrous tissue." These very marked vessel changes described by Dawson are more severe than those usually seen in nodules as described by others or those which comprise the present study. Klinge (24) in discussing the pathology of rheumatic fever does not describe the subcutaneous nodule separately from the myocardial lesion, since he believes the two are simply tissue variations of a single basic alteration of structure, the result of the rheumatic fever virus. "The rheumatic nodule is not the first or most important alteration, but is a stage of higher development. It is formed on the groundwork of a primary degenerative alteration of the connective tissue, especially of the interfibrillar and intercellular substance. A degeneration of the connective tissue with swelling of the

interfibrillar substance is the most important and ubiquitous part in the development of the rheumatic lesion." He describes at some length the connective tissue changes seen in the early lesion of rheumatic fever, and emphasizes the degeneration of collagen with later fibrin deposition and edema and states that only lymphocytes and polymorphonuclear leukocytes may be seen in the early stage of development. In his description of older lesions he describes the granuloma cells developing from the fibrocytes in the region of the lesion. McEwen (25) used the supravital technic in an attempt to identify more accurately the cells in subcutaneous nodules, but concluded only that they were not clasmatoocytes or epithelioid cells, and that the giant cells were of neither the tuberculous nor the foreign body type.

From the above review of the literature it is apparent that there are distinct variations in description and yet in most of them there are three general types of change to be noted. First, there is an alteration of collagen, associated frequently with fibrin deposition; second, there is a cellular infiltration and proliferation of large cells some of which resemble fibroblasts; and third, there is present increased vascularization of the surrounding tissue with concurrent vessel damage. These are the three basic types of tissue reaction seen in the subcutaneous nodule, and it is our purpose to correlate the variations of these three types of change with the clinical age and type of nodule to determine if many of the discrepancies in the literature are not simply nodules obtained in different stages of their evolution or regression.

#### MATERIALS AND METHODS

The nodules studied were with two exceptions biopsy specimens. In each instance the nodule was carefully dissected out after regional nerve block anesthesia had been instituted to prevent distortion of the tissue by the local injection of an anesthetic. The specimens were fixed in most instances in Zenker's fixative, but in some cases Zenker-Formol was used. The blocks were paraffin imbedded, sectioned at 6 micra, and representative sections from all blocks stained with Delafield's hematoxylin-eosin and with Mallory's aniline blue stain. In addition, sections from most of the specimens were stained with Mallory's phosphotungstic acid hematoxylin and Maximow's eosin azur II. Van Gieson's stain and Weigert's elastic tissue stain were also applied to representative sections.

The nodules were removed in the following time intervals from their clinical appearance:

- |                                     |    |
|-------------------------------------|----|
| 1. Present less than 1 month .....  | 6  |
| 2. Present 1 to 3 months .....      | 10 |
| 3. Present more than 3 months ..... | 7  |

A summation description of each group is presented. Since the subcutaneous nodule is frequently made up of a number of minute lesions in various degrees of injury or organization, the structure of the large majority of lesions is interpreted to represent the histopathology of the clinical nodule.

When the clinical variations of the appearance, characteristics, and duration of nodules is considered, definite differences of histopathology are to be expected. In an attempt to ascertain the basis for these differences, we have accumulated a small series of nodules, the clinical ages of which are known. This was done in order to define, if possible, the progressive histological structure of the subcutaneous nodule.

#### *Subcutaneous nodules present less than 1 month*

Nodules of this age group have characteristic features which exhibit a general basic similarity although there may be some variation of finer structure. Histologically there is in most instances widespread edema of the intercellular and interfibrillar spaces which is not sharply demarkated from the surrounding tissue (Figures 11 and 12). This edema is usually present to some extent throughout the gross lesion, but more commonly there are areas of varying size of more conspicuous accumulation of fluid separating intervening altered tissue. This loose edematous structure accounts for the fact that, in the gross, newly detected nodules are usually relatively soft to palpation and are ordinarily poorly circumscribed. There are several changes which are fundamentally the same in all nodules of this group, although the structure may vary from one specimen to another. This in part depends on the density of the tissue concerned.

1. *Collagen change.* The collagen in all of the young specimens has a uniform change of staining reaction. It is brightly eosinophilic staining with Delafield's hematoxylin-eosin, fails to take Mallory's aniline blue, and may or may not take a specific fibrin stain. In agreement with Klinge (24) there is to be found an alteration of the collagen fibrils themselves as well as a change in the interfibrillar and intercellular spaces with the deposition of a substance which in most instances takes a fibrin stain. The modified collagen has



exhibited four different trends depending in part at least on the density of the tissue in which the lesion is located. (a) The collagen in dense connective tissue may form wide, compact, acellular, homogeneous, acidophilic bands in which fibrils cannot be made out by ordinary stains (Figure 1). This tissue is usually separated by more or less fluid. In the gross this is usually a small, firm, discrete nodule. (b) There may be widespread necrotization and fragmentation and even complete dissolution of collagen resulting in edema filled spaces with large fragments of necrotic, gelatinized, acellular, eosinophilic collagen (Figure 11). This type of nodule is usually large and soft even though it is in dense connective tissue. (c) The changed connective tissue may be arranged as coarse, intensely eosinophilic strands which tend to form a distinct concentric lattice work widely dispersed by edema, usually at a little distance from blood vessels, and richly infiltrated with cells. This type of nodule is usually large, soft, and situated in loose, irregular subcutaneous tissue (Figures 4, 8, and 12). (d) The collagen change may manifest itself as a fine, irregular, interweaving, fragmented network of loose necrotic fibrillar collagen with or without fibrin deposition (Figure 3). These areas are usually small, scattered, multiple, and situated at a short distance from the commonly damaged vessels. This type of reaction is usually found in loose subcutaneous tissue.

2. *Vessel change.* At the periphery of, or traversing the areas of necrotic tissue in these younger nodules, there are usually a number of small arteries, veins, and capillaries. Practically all the vessels, including capillaries, exhibit thickened, proliferative, rounded or almost cuboidal, deeply basophilic staining endothelium (Figures 3, 4, and 5). Many of the larger vessels show an intima thickened by subendothelial proliferation (Figure 8). Vacuolization and swelling of the subintimal cells is not uncommon with resulting marked narrowing of the lumen. The alterations in the media, when present, consist mostly of vacuolization and general diffuse swelling of the cells of the muscularis (Figures 4 and 8). Many of the vessels have a moderate polymorphonuclear leukocytic and lymphocytic infiltration involving all their layers and extending

into the perivascular tissues. Thrombus formation in the vessels has not been observed. In general, the vessel changes are not as severe as those described by Dawson and Boots (23).

3. *Cellular reaction.* In this early stage, there is usually a very definite perivascular proliferation and infiltration of the surrounding tissues. This is less marked in the small compact nodules and more general in the larger acute edematous specimens. The most marked proliferation is on the part of large, pale staining perivascular cells, the cytoplasm of which cannot be clearly defined by any of the stains used. The nuclei of these are large and oval with a pale staining, widely dispersed chromatin network. There is a large round nucleolus demonstrable by Maximow's eosin azur II stain. The exact nature of these cells cannot be ascertained, but they appear to be of the undifferentiated mesenchymal type (Figures 4 and 5). Surrounding and merging with this perivascular mantle of cells, and invading the border of the necrotic tissue are numerous scattered, more distinct, irregularly shaped mononuclear cells. These cells have a pale to deeply staining large reticular nucleus and a scanty, deeply basophilic staining, dusty, non-granular cytoplasm with one or more blunt, indistinct cytoplasmic processes, demonstrable by Mallory's phosphotungstic acid hematoxylin stain. A single large nucleolus may be seen with Maximow's eosin azur II stain. Some of these cells have much more distinct numerous long branching cytoplasmic processes demonstrable by the Mallory phosphotungstic acid hematoxylin stain, and appear to be altered fibroblasts (Figures 3 and 5). Multinuclear cells are uncommon, but when present they have the same general type of nucleus as the cells just described with either pale or deeply staining scanty and poorly outlined cytoplasm with or without branching, blunt cytoplasmic processes. In general, none of the above cells is strikingly similar to the Aschoff cell as seen in the myocardium, particularly in the appearance of the nuclei. Among these invading cells and scattered throughout the perivascular areas are many lymphocytes and a fair number of polymorphonuclear leukocytes and phagocytic wandering cells.

*Subcutaneous nodules present 1 to 3 months*

The characteristics of the nodules of this group substantiate the impression that there is a progressive organization of the lesions seen in the younger specimens. Consequently edema is insignificant, the vascularization is much more intense, and the lesion much more richly cellular than in younger nodules (Figure 6). As a result of this, grossly, nodules of this age group although they may vary considerably in size, are usually firm to palpation and ordinarily well circumscribed. As is the case with younger nodules there are three general types of change to be seen.

1. *Collagen change.* The foci of altered collagen are usually smaller and situated at a distance from invading blood vessels (Figures 6, 9, and 10). The staining affinity instead of having one type of reaction may stain either red or blue with hematoxylin and eosin stain and may appear to have in many instances basophilic granular precipitated material superimposed or in the tissue. The material does not take Mallory's aniline blue and frequently does not show an affinity for the fibrin stain. As may be expected from the several types of collagen alteration seen in the younger specimens, there is more than one type of collagen focus to be seen in specimens of this group. (a) The necrotic collagen may be present as compact fibrillar or amorphous acidophilic or basophilic staining plaques usually with detectable fibrin and invaded at the periphery by an intense cellular reaction (Figure 6). (b) The altered collagen may consist of small foci of fragmented, irregular, anastomosing, amorphous or beaded, eosinophilic or basophilic staining, interweaving strands of irregular size and shape. This material usually takes neither the aniline blue stain nor a fibrin stain. Invading these foci are densely packed cells of the type to be described shortly, similar in type to that seen in Figure 3.

2. *Vessel change.* There is a very definite vascularization of the whole nodule with a marked increase in the number of arterioles, venules, and capillaries present. The endothelium of most of the vessels has the same characteristics as those seen in the vessel linings of younger specimens, but they exhibit in addition marked vacuolization of the endothelial lining cells. In many instances

there is proliferation and thickening of the subintimal tissue with the deposition of an amorphous intercellular material which takes a diffuse blue color with Mallory's aniline blue stain (Figures 6 and 8). The elastica is not infrequently interrupted and fibrillar. Definite injury to the media is evidenced by swelling of muscularis cells and hyalinization with replacement of fibrous tissue in some instances. These changes result in marked narrowing of the lumen particularly of the arterioles. No actual thromboses or canalization of vessels have been seen. Lymphocytes are not uncommonly seen infiltrating the vessel walls, but polymorphonuclear leukocytes are rare.

3. *Cellular reaction.* About most of the vessels there is only a slight proliferation on the part of undifferentiated perivascular cells, similar to that seen in the younger nodules (Figures 6, 8, or 9). In general the perivascular cells are more distinct, the cytoplasm is clearly outlined, the nuclei are more deeply staining and reticular, and do not appear as actively proliferating as in younger nodules. About this irregular area of perivascular cells there is a gradation into closely radially arranged more mature cells having definite, sharply outlined basophilic staining, dusty, non-granular cytoplasm. The nuclei are dark, irregularly staining or reticular, and either single or multiple. Long, branching, intertwining cytoplasmic processes may be seen in sections stained with Mallory's phosphotungstic acid hematoxylin. There is definitely an increase in normal young fibrous tissue about the blood vessels and numerous normal looking collagen fibrils may be seen between the cells just described. As the areas of necrosis are approached, the collagen fibers become less numerous and less distinct, the cells tend to be more closely packed, and the processes of individual cells are made out with much greater difficulty. The cytoplasm is more deeply basophilic staining, is less abundant, is more poorly outlined, and cytoplasmic processes are few or absent. Multinuclear cells are common though in no instance are they numerous. These basophilic cells are similar in character though much more numerous than in younger lesions. The infiltration by polymorphonuclear leukocytes is minimal, phagocytic cells are few, and the lymphocytic reaction only moderate. The general appearance is that

of a progressive organization of the larger areas of collagen change seen in many of the younger nodules.

*Subcutaneous nodules present more than 3 months*

The characteristics of the nodules in this age group indicate a further organization of the previously described lesions.

1. *Collagen change.* There is a continuation of fibrous tissue organization with large areas of normal looking dense collagen for considerable distances from vessels (Figures 10 and 15). There are in some instances plaques of dense amorphous eosinophilic or basophilic material, the borders invaded by intensely basophilic cells and situated in large surrounding areas of normal looking young fibrous tissue (Figure 10). More common are small foci of amorphous or beaded, irregular, anastomosing, deeply blue staining strands located at a considerable distance from blood vessels (Figure 15). These represent the remnants of incompletely organized areas of injury noted in the younger nodules.

2. *Vessel change.* The vascularity of the lesion is still apparent but the number of capillaries is very definitely decreased in comparison with younger nodules. In these nodules the thickened basophilic endothelium, the narrowed lumen, the thickened intima, and fibrosed media persist in the medium sized vessels as do the endothelial changes in the precapillaries and capillaries. There is little or no cellular infiltration of the vessel walls although there is usually a slight lymphocytic reaction about the vessels.

3. *Cellular reaction.* In most sections there is practically no proliferation or extension of perivascular cells into the surrounding tissue. There is a large amount of proliferation of fibrous tissue which near the vessels is not very cellular, but nearer the remaining necrotic areas is more cellular. Immediately about the foci, the structure is like that at the periphery of the foci in the younger nodules with numerous closely packed basophilic cells with numerous intercellular fibrils. The cells in and around the necrotic areas are of the same type as those seen about the necrotic foci in nodules of the second group, although they are in general more intensely basophilic with both

the nucleus and cytoplasm taking a deep hematoxylin stain. Cytoplasmic processes are few and blunt, but normal intercellular collagen fibrils may be made out. Surrounding these peri-focal cells are numerous more normal looking, branching, active fibroblasts with large amounts of fibrillar intercellular collagen. The cells in the more mature connective tissue are not numerous and are elongated, normal, non-proliferative, inactive fibroblasts. There is a gradual gradation in characteristics from normal looking fibroblasts to the typical basophilic mononuclear and multinuclear cell which is so characteristic of the nodule and raises the question of their origin and function.

It should be noted that there were two nodules on which could be made no specific tissue diagnosis from their histological structure. They appeared to be more of a foreign body organization of nonspecific character.

The foregoing descriptions have been presented in an attempt to define the structure of the subcutaneous nodule and to gain some insight into the evolution and regression of these structures in subjects with rheumatic fever. That there is a considerable variation of structure is evident, but it seems likely that these variations may be explained on the basis of different stages in their evolution, and probably also on differences of anatomical location, i.e., whether they evolved in dense or loose connective tissue.

*The structure of induced subcutaneous nodules in subjects with rheumatic fever*

In the previous report (1) it has been demonstrated that the injection of blood into the deep subcutaneous tissues of subjects with rheumatic fever in certain stages of the disease is followed by a definite nodular reaction after about one to two weeks. It has further been stated that these nodules are grossly indistinguishable from the spontaneously occurring nodules so frequently seen in this disease. A comparison of the histological structure of these lesions with those of spontaneously occurring rheumatic fever nodules will be presented.

Of 37 nodules which were observed to appear following the procedures described in the preceding report, 19 were biopsies. They were removed carefully after regional anesthesia and were fixed,

sectioned, and stained in the same manner as were the spontaneously occurring nodules.

The nodules were removed after the following intervals from the time of their clinical detection:

1. Present less than 1 month .....	15
2. Present 1 to 3 months .....	4
3. Present more than 3 months .....	0

#### *Induced nodules present less than 1 month*

About half of the nodules in this group were removed after being present about 2 weeks; the other half after nearly 4 weeks. The latter in most instances presented the more characteristic histological structure. There is a considerable variation of structure in this group of nodules, but there is the same general tissue reaction noted in spontaneous nodules. As is the case with most young spontaneous nodules, edema is usually quite marked, separating the more common wide bands of necrotic connective tissue (Figures 13, 14, and 19). The fluid filled spaces have more amorphous precipitated material than do most spontaneous nodules, and there may be remaining old red blood cells in the same areas.

1. *Collagen change.* The alteration of structure and the staining reaction of the collagen in these induced lesions is similar to that seen in spontaneously occurring nodules of similar age. The area of the altered collagen is usually large and even macroscopic in size. The necrotic collagen usually forms wide bands of intensely eosinophilic homogeneous or fibrillar material, separated by fluid spaces containing considerable precipitated amorphous protein (Figure 19). In some instances there is found at a short distance from blood vessels an intensely eosinophilic staining irregular lattice work structure, identical to that seen in some spontaneous lesions (Figures 13 and 14). The altered collagen does not stain by Mallory's aniline blue and may or may not take a fibrin stain. The condition of the collagen fibrils and the interfibrillar spaces are indistinguishable from the changes seen in spontaneous nodules except for the presence of more amorphous precipitate.

Centrally in these large areas of collagen necrosis there is usually a fair amount of the above mentioned precipitate and sometimes old red blood cells and fibrin deposition. In these same

central areas there are few if any remaining blood vessels and little if any cellular infiltration; these latter are limited usually to the periphery of the lesion (Figure 19).

2. *Vessel change.* Invading the periphery of and in some cases traversing the lesion may be seen numerous proliferating, invading blood vessels and many dilated capillaries. There is the same markedly thickened basophilic endothelium seen in spontaneous nodules, and many of the vessels exhibit marked vacuolization of the intima with subintimal and medial swelling resulting in a definite decrease in the lumina of the vessels (Figures 16 and 20). There is in most instances a very definite polymorphonuclear leukocytic and lymphocytic infiltration of all of the layers of the vessel walls as well as of the perivascular tissues, appearing occasionally almost as a periarteritis. The vascular damage in these induced nodules is similar to, if not identical with that seen in spontaneous nodules, but in general is less severe.

3. *Cellular reaction.* Immediately surrounding the vessels in many nodules there is a definite proliferation of pale staining mononuclear cells which have very vague or indistinguishable cytoplasm, by any method of staining used. The nuclei are very large, oval, and pale staining with a widely dispersed chromatin network. A large purplish eccentrically placed nucleolus may be demonstrated by the polychrome stain. This perivascular proliferation in many instances forms a definite mantle about the vessels and is identical with the reaction seen about blood vessels in some young spontaneous nodules (Figures 20, 21 and 24). These seem to be undifferentiated perivascular mesenchymal cells. There is an associated moderate, perivascular, polymorphonuclear leukocytic and lymphocytic infiltration with a variable number of phagocytic cells. In the more typical nodules about this perivascular reaction there is a more extensive cellular response consisting of basophilic staining, elongated cells which have a large irregularly staining almost reticular nucleus. The cytoplasm is usually scanty, has an indefinite outline, is commonly basophilic and may or may not have cytoplasmic processes demonstrable by Mallory's phosphotungstic acid stain. Their long axes are in the direction of and in some instances encroaching on the border of the necrotic col-

lagen. These cells are indistinguishable from those seen in spontaneously occurring nodules. Occasionally, multinuclear cells of similar character may be seen (Figures 16, 20, 21, and 24). In the more atypical nodules there are fewer of these cells and more phagocytic wandering cells (Figure 17). Lymphocytes, polymorphonuclear leukocytes, and phagocytes are more common in most of the induced nodules than in spontaneous nodules, and occasional foreign body giant cells are seen. In 4 instances the tissue reaction seems to be a nonspecific type of reaction to a foreign material except for the collagen change, the edema, and the vessel alteration. In this respect it is to be recalled that in two spontaneously occurring nodules it was impossible to make a specific tissue diagnosis histologically.

#### *Induced nodules present 1 to 3 months*

All of the induced nodules in this group were removed after an interval of 2 to 3 months had elapsed since their clinical detection. Since they were in every detail indistinguishable from spontaneously occurring nodules of a similar age it is unnecessary to describe them separately (Figures 18, 22, and 23).

One induced nodule appeared in the control group. No definite diagnosis of rheumatic fever could be made in this patient, although the evidence against it was not absolutely convincing. Histologically, this nodule appeared to be simply an old organizing fibrosis of nonspecific character.

#### DISCUSSION AND CONCLUSIONS

From the review of the literature and from the descriptions given above it is evident that considerable variations of structure may be seen in the subcutaneous nodules of rheumatic fever. This tissue alteration may vary from a very characteristic structure to a microscopic picture resembling simply foreign body or necrotic tissue organization with few typical changes to permit a specific tissue diagnosis. An attempt has been made to explain these variations of microscopic structure from a consideration of the clinical age of the nodules studied and the density of the tissue in which they are located. This has been

difficult and only roughly approximate since the nodule is made up of submiliary and microscopic areas in different stages of organization. Therefore, only the trend of the majority of lesions may be considered to represent the pathological structure of the clinical nodule.

In spite of the difficulties encountered in studying these structures, it is believed that the histological evolution and regression of the subcutaneous nodule has been approximated in the small series studied. There is apparently first an alteration in the structure of collagen with the resulting edema formation and deposition of fibrin-like material. Concurrent with or shortly after this there is a vascular damage and a polymorphonuclear leukocytic and lymphocytic cell infiltration with a proliferation on the part of primitive perivascular mesenchymal and other cells which invade the borders of the altered collagen foci. Subsequent to this there is a gradual organization progressing from the perivascular areas toward the centers of the necrotic foci. During the course of this organization there results a typical type of cellular reaction consisting of closely packed, radially arranged, basophilic staining mononuclear and multinuclear cells which have staining reactions somewhat similar to fibroblasts and which are apparently capable of depositing intercellular collagen. The outcome is a progressive organization to a normal fibrous tissue replacement of the lesion. From this it appears that there is a definite progression of structure throughout the evolution and regression of the lesion.

Histologically, the structure of the artificially induced nodules is found to be very similar to, if not identical with that of spontaneously occurring nodules. There are, however, certain types of alteration in the histopathology of induced nodules which are inherent in the method of traumatization used and which distort the minute structure whether or not the tissue reaction is similar to that of the spontaneously occurring nodule. In the method used, there resulted a considerable damage and distortion of the subcutaneous connective tissue structure with resulting interference with tissue nutrition. Consequently, in the induced nodules there are the following three points

in common and different from most of the spontaneously occurring nodules. First, the areas of collagen alteration are much larger and the strands of collagen are considerably wider than is the case with most spontaneous nodules. Second, an appreciable amount of blood is introduced intercellularly which results in a larger phagocytic cell response than is the case in most of the young spontaneous nodules; this is most apparent in the younger nodule. Third, there is a considerable amount of intercellular and interfibrillar amorphous precipitate in these lesions apparently the result of introducing large amounts of blood into the tissue spaces. These differences are present whether or not the vascular and cellular response is similar to that of the spontaneous nodules, and are significant only in the nodules in the younger age group. Although these artificial characteristics usually allow for a differentiation between young induced and young spontaneous nodules, the type of tissue response is in most instances similar and in some cases indistinguishable. No difference of structure can be determined between induced and spontaneous nodules in the older age group.

The results indicate that not only are the induced nodules clinically indistinguishable but pathologically also there is a great similarity of structure between these and spontaneously occurring nodules of similar age.

To Doctors John W. Spellman and Grantley W. Taylor, of the House of the Good Samaritan Surgical Staff, we wish to express our appreciation for performing the biopsies. The authors are indebted to Doctors Granville F. Bennett and Shields Warren for helpful suggestions and criticism.

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## LEGENDS TO FIGURES

FIG. 1. SPONTANEOUS SUBCUTANEOUS NODULE REMOVED 7 DAYS AFTER ITS APPEARANCE. H & E.  $\times 135$ .

Right upper portion of section consists of broad fragmented bands of intensely eosinophilic dense altered connective tissue separated by edema. Extending diagonally across the center of the section may be made out a radially invading layer of thin, elongated, closely packed, poorly outlined hasophilic cells. Infiltration by other types of cells is minimal.

FIG. 2. SPONTANEOUS SUBCUTANEOUS NODULE REMOVED 3 MONTHS AFTER ITS DETECTION, AREA OF ACUTE CHANGE WITHIN AN OLDER NODULE. H & E.  $\times 135$ .

In the right lower portion may be made out several small vessels which are normal except for dilatation. The perivascular tissue is very edematous with a slight lymphocytic infiltration. In the upper left portion of the section are wide homogeneous bands of deeply eosinophilic collagen in which the normal collagen structure is masked. There is no characteristic cellular reaction in this section.

FIG. 3. SPONTANEOUS SUBCUTANEOUS NODULE REMOVED 7 DAYS FROM ITS CLINICAL APPEARANCE. H & E.  $\times 150$ .

In the left lower portion are a number of small dilated capillaries with definite perivascular fibrosis. The upper central portion is occupied by a focus of fragmented necrotic collagen with considerable edema. Lymphocytes and large basophilic mononuclear cells may be seen invading the periphery of the focus. The cellular nature of the surrounding fibrous tissue indicates the probable existence of an older subclinical nodule for some time.

FIG. 4. SPONTANEOUS SUBCUTANEOUS NODULE REMOVED 5 DAYS AFTER ITS APPEARANCE. H & E.  $\times 135$ .

This section demonstrates well the small vessel damage, the perivascular mononuclear cell proliferation, and the irregular lattice work structure which is seen in some nodules. The cellular reaction is typical but not marked for an early nodule of this type.

FIG. 5. SPONTANEOUS SUBCUTANEOUS NODULE REMOVED 7 DAYS AFTER ITS DETECTION. H & E.  $\times 150$ .

To the right are seen several small vessels which have somewhat thickened endothelial lining cells. There is definite edema of the perivascular tissues with a moderate lymphocytic infiltration. The larger elongated deeply basophilic cells are discernible with their radial arrangement at the border of the necrotic edematous collagen at the extreme left.

FIG. 6. SPONTANEOUS NODULE REMOVED 3 MONTHS AFTER ITS CLINICAL DETECTION. H & E.  $\times 175$ .

There is definite vascularization of the tissue in the left center with thickening of the walls of the small vessels and thickening of the endothelium. The young loose fibrous tissue in the whole area is apparent. The large mononuclear and multinuclear cells are numerous and the intercellular collagen is easily seen. This is a progression of the organizing process.

FIG. 7. SPONTANEOUS SUBCUTANEOUS NODULE 5 MONTHS OLD. H & E.  $\times 135$ .

Upper portion of section consists of normal dense irregular connective tissue. The left lower portion is a very cellular fibrous tissue having no distinguishing cellular characteristics. This is a late stage of fibrosis in a subcutaneous nodule.

FIG. 8. SPONTANEOUS SUBCUTANEOUS NODULE REMOVED 3 MONTHS AFTER ITS APPEARANCE. H & E.  $\times 275$ .

This section is of an area of more acute damage in a nodule constituted mainly of tissue consistent with an older nodule. The vascular damage is the most obvious alteration with thickening of both the endothelial lining and the vessel walls with swelling, subintimal proliferation, and polymorphonuclear leukocytic infiltration. The perivascular edema and cellular infiltration with polymorphonuclears, lymphocytes, phagocytic cells, and necrotic collagen strands may be made out in the extreme upper portion. There is no characteristic cellular reaction.

FIG. 9. SPONTANEOUS SUBCUTANEOUS NODULE 3 MONTHS OLD. H & E.  $\times 300$ .

Moderately high power magnification to show the cellular detail. Fine long normal intercellular fibrils of collagen may be made out between the large rather closely packed basophilic staining cells.

FIG. 10. SPONTANEOUS SUBCUTANEOUS NODULE OF 4 MONTHS DURATION. H & E.  $\times 135$ .

Lower portion of section is made up of dense irregular connective tissue; the mild reaction about the small vessels is to be noted. The middle portion is made up of rather cellular proliferating fibrous tissue with a few of the typical basophilic cells invading the necrotic material.

FIG. 11. SPONTANEOUS SUBCUTANEOUS NODULE REMOVED 7 DAYS AFTER ITS CLINICAL APPEARANCE. H & E.  $\times 25$ .

Low power view to show the almost cystic character of the damaged areas of collagen. The upper center portion is a very edematous area of fragmented necrotic collagen. There are several small vessels traversing the lower half of the section with a perivascular cellular reaction.

FIG. 12. SPONTANEOUS SUBCUTANEOUS NODULE REMOVED 5 DAYS AFTER ITS APPEARANCE. H & E.  $\times 25$ .

Low power view to show the widespread edema of the tissue with the numerous irregular strands of intensely eosinophilic altered collagen.

FIG. 13. INDUCED SUBCUTANEOUS NODULE REMOVED 10 DAYS AFTER ITS APPEARANCE. H & E.  $\times 45$ .

Low power view to show the widespread edema of the tissue with the characteristic lattice type of condensed eosinophilic staining necrotic collagen strands so frequently seen.



FIG. 14. BIOPSY OF INDUCED SUBCUTANEOUS NODULE 3 WEEKS AFTER ITS APPEARANCE. H & E.  $\times 35$ .

Low power view to demonstrate the deeply staining irregular fragmented collagen strands in the center. Edema is not so marked as in younger specimens.

FIG. 15. SPONTANEOUS SUBCUTANEOUS NODULE OF 4 MONTHS' DURATION. H & E.  $\times 135$ .

Another area of nodule as shown in Figure 10. The abundant cellular fibrous tissue with a slight lymphocytic reaction is the only residuum of the organizing nodule.

FIG. 16. BIOPSY OF INDUCED SUBCUTANEOUS NODULE 3 WEEKS FROM THE TIME OF ITS DETECTION. H & E.  $\times 150$ .

The general edema of the section is evident. Definite alteration of small vessel structure may be made out and the slight proliferation of pale staining perivascular cells may also be seen. The presence of large deeply staining cells may be seen as well as numerous lymphocytes and phagocytes.

FIG. 17. INDUCED SUBCUTANEOUS NODULE REMOVED 4 WEEKS AFTER ITS APPEARANCE. H & E.  $\times 135$ .

Necrotic fragmented collagen may be seen above. In the central portion of the section are lymphocytes, a few phagocytes, and a fair number of large basophilic cells similar to those seen in young spontaneous nodules.

FIG. 18. INDUCED SUBCUTANEOUS NODULE REMOVED 8 WEEKS AFTER ITS APPEARANCE. H & E.  $\times 125$ .

The necrotic deeply staining, fragmented collagen may be made out to the right. The alteration of vessels is definite but not marked, but the cellular reaction invading the collagen is typical and indistinguishable from that seen in spontaneous nodules.

FIG. 19. INDUCED NODULE REMOVED 3 WEEKS AFTER ITS DETECTION. H & E.  $\times 45$ .

Low power field to show the widespread damage that may be present in some induced nodules. Red blood cells may be made out in the fluid-filled spaces. Relatively normal, dense connective tissue may be made out to the extreme left.

FIG. 20. BIOPSY OF INDUCED SUBCUTANEOUS NODULE 2½ WEEKS FROM CLINICAL APPEARANCE. H & E.  $\times 275$ .

The vascular change is evident with a definite lymphocytic infiltration of the walls and thickened, swollen

endothelium and media. The surrounding edema with the "lattice work" collagen change above is apparent. Lymphocytes and phagocytes are definite and a few large reticular nucleated, basophilic staining mononuclear cells may be made out.

FIG. 21. INDUCED SUBCUTANEOUS NODULE REMOVED 4 WEEKS AFTER ITS APPEARANCE. H & E.  $\times 250$ .

This section demonstrates the characteristic small vessel change with surrounding perivascular proliferation, edema, and lymphocytic and phagocytic cell infiltration. Large elongated deeply basophilic staining cells, which cannot be differentiated from those seen in spontaneous nodules, are also present. In the left upper corner are irregular fragmented strands of collagen.

FIG. 22. BIOPSY OF INDUCED SUBCUTANEOUS NODULE 2½ MONTHS FROM TIME OF APPEARANCE. H & E.  $\times 150$ .

Section shows fibrosis about borders with centrally an area of cellular reaction, encroaching on a few strands of deeply basophilic staining necrotic collagen. The cells involved and general structure cannot be distinguished from those seen in spontaneously occurring nodules of similar age.

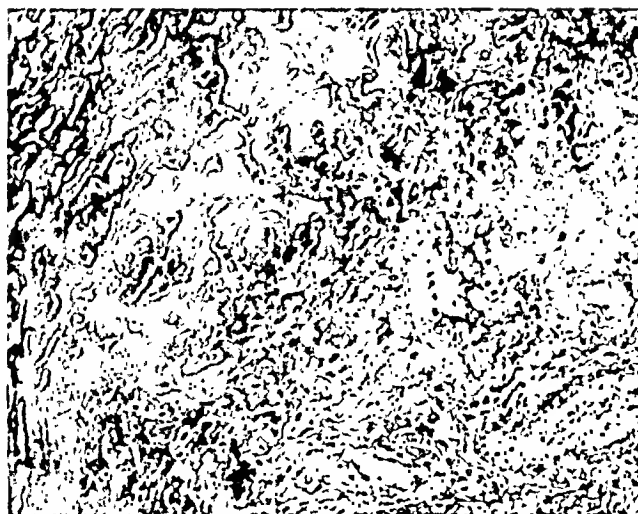
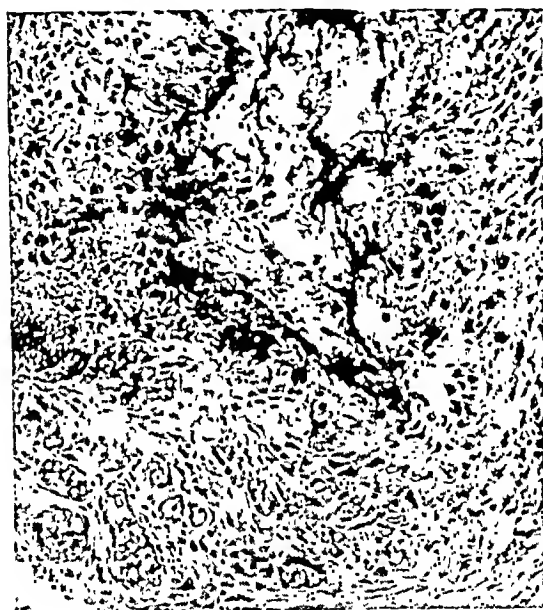
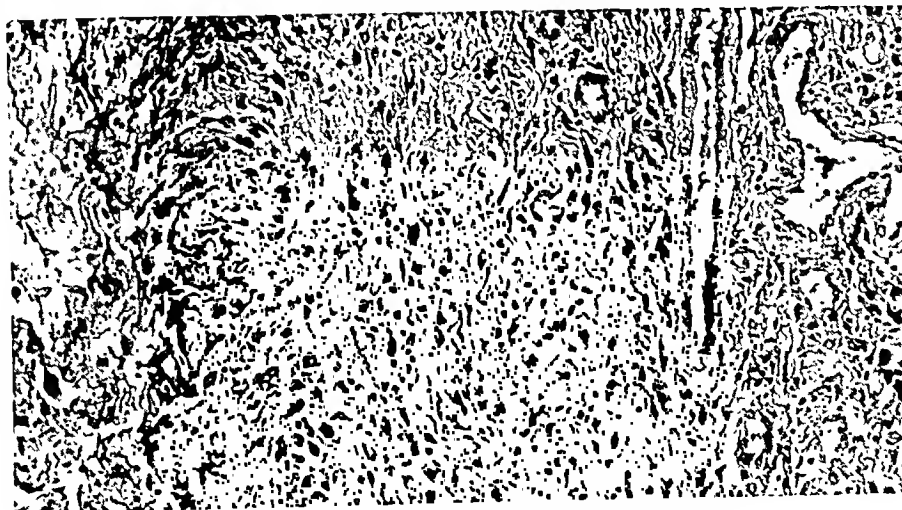
FIG. 23. INDUCED SUBCUTANEOUS NODULE REMOVED 4 WEEKS AFTER ITS DETECTION. H & E.  $\times 275$ .

In the left upper corner is degenerated, eosinophilic, fragmented collagen. The greater portion of the section is made up of closely packed large basophilic staining cells with indistinct cytoplasmic processes. These cells are in character indistinguishable from those found in spontaneous nodules. There is as yet little intercellular collagen present.

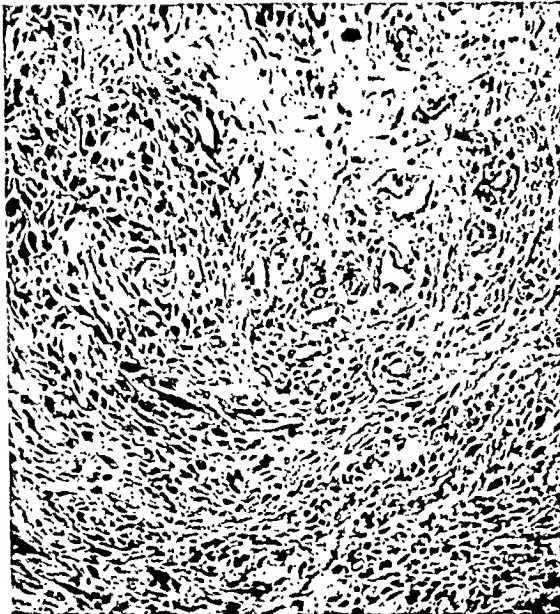
FIG. 24. INDUCED SUBCUTANEOUS NODULE REMOVED 3 WEEKS AFTER ITS CLINICAL APPEARANCE. P. T. A. H.  $\times 135$ .

The widespread edema is evident. The small vessel change can be easily made out as can the collagen change in the upper and left field. The cytoplasmic processes of the large basophilic staining cells are well shown. These cells cannot be differentiated from those seen in spontaneously occurring nodules. Lymphocytes and phagocytes are both present. The general structure is identical to that seen in younger spontaneous lesions.



**FIG. 1****FIG. 2****FIG. 3****FIG. 4****FIG. 5**

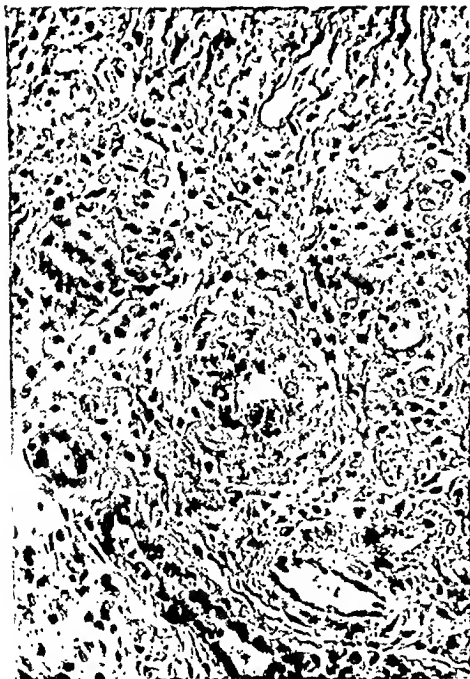
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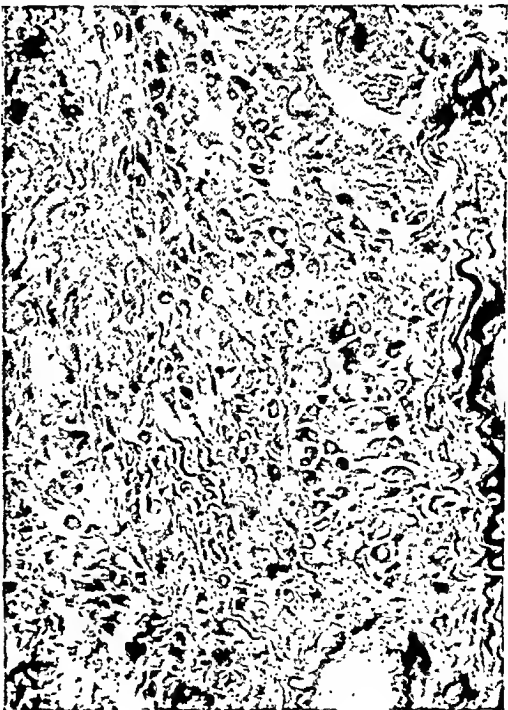
**FIG.6**



**FIG.7**

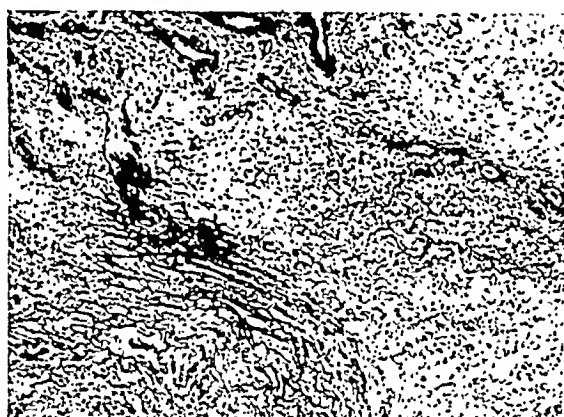
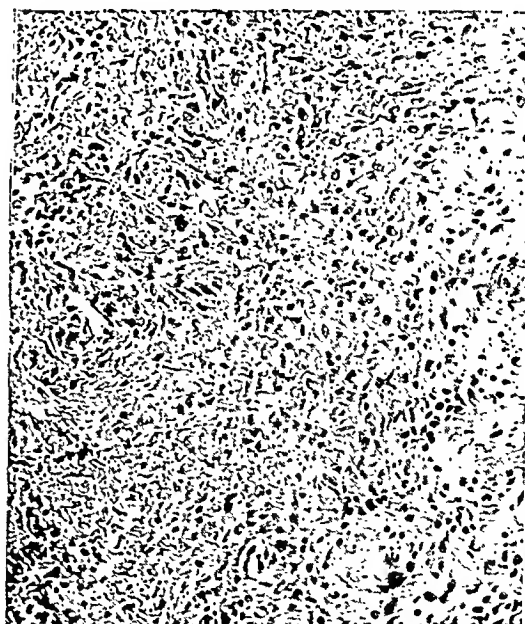


**FIG.8**

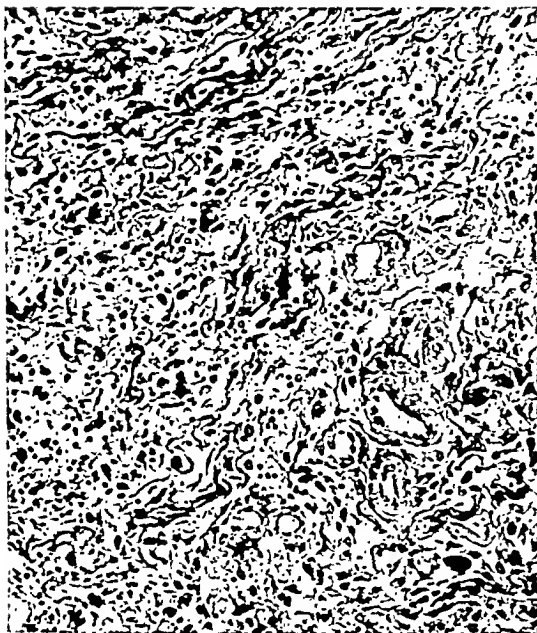
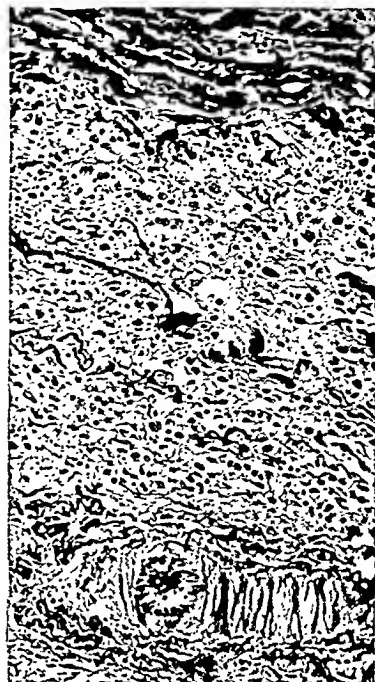


**FIG.9**

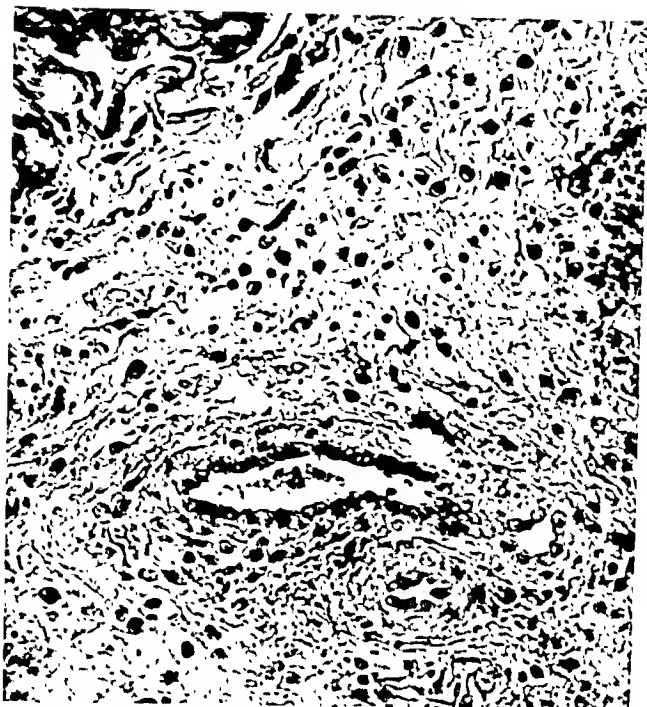
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**FIG. 10****FIG. 11****FIG. 12****FIG. 13****FIG. 14****FIG. 15**

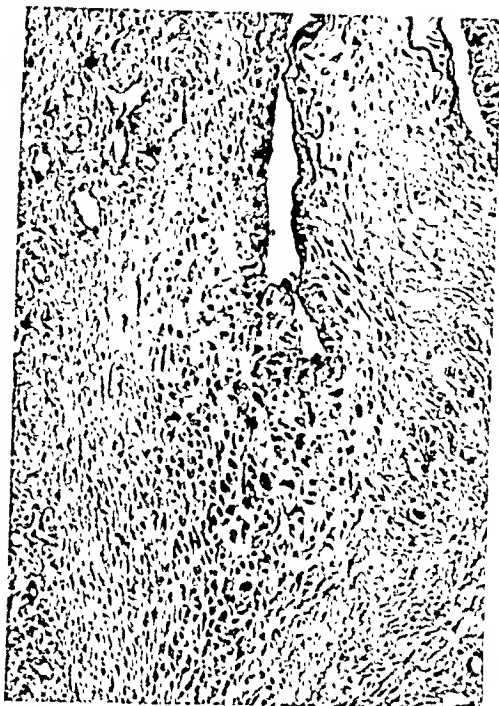
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**FIG.16****FIG.17****FIG.18****FIG.19****FIG.20**

SEE PAGE 139 FOR LEGENDS.



**FIG.21**



**FIG.22**



**FIG.23**



**FIG.24**

SEE PAGE 139 FOR LEGENDS.



# DEVELOPMENT OF ANTIFIBRINOLYTIC PROPERTIES IN BLOOD OF PATIENTS WITH RHEUMATIC FEVER, CHRONIC INFECTIVE ARTHRITIS AND BACTERIAL ENDOCARDITIS<sup>1</sup>

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(Received for publication September 8, 1936)

The ability of the hemolytic streptococci of the beta type to dissolve human fibrin clot has been demonstrated by Tillett and Garner (1). They have also found (2) that the blood of patients convalescent from acute hemolytic streptococcal infections was highly resistant to the fibrinolytic principle. This antifibrinolytic property of the blood has been tested by several authors in various infections due to hemolytic streptococci. Tillett (3) found that the antifibrinolytic factor was demonstrable in the blood of seventy-five per cent of patients who had recovered from acute streptococcal infections. In rheumatic fever the antifibrinolytic property seemed to follow the acute upper respiratory infection. Hadfield, Magee, and Perry (4) reported that the plasma clot was resistant during the active stage of the disease in rheumatic fever. The antifibrinolysin reaction has also been used by Myers, Keefer and Holmes (5), who found positive reactions in rheumatic fever, but negative reactions in rheumatoid arthritis.

In a previous paper (6) the author reported the results of antifibrinolysin tests in which Tillett and Garner's technic (1) was used. The results were graded according to the rapidity with which dissolution took place. Each plasma was tested against 5 strains of streptococci with varying fibrinolytic ability; only one dilution of active culture was used. With this technic we were able to demonstrate positive antifibrinolysin reactions in 5 cases of acute polyarthritis, while 5 others were negative in the active stage of the disease. In a few cases of rheumatoid arthritis we also found resistance to the fibrinolytic principle. We therefore decided to try another technic in order, if possible, to demonstrate weaker positive reactions.

## MATERIALS AND METHODS

We have studied 7 cases of acute polyarthritis, 24 cases of chronic infective polyarthritis, and 5 cases of bacterial

<sup>1</sup> This study was made possible by the aid of the Else and Marie Mustad Fund.

endocarditis. Most of the patients have been under treatment at the Medical Department B, Rikshospitalet. Some tests were carried out with samples of blood sent from other departments and hospitals. In order to state the "normal" reaction we have performed tests on 39 healthy adults, chosen among students, technicians and members of the hospital staff. In this group we selected those who were healthy at the time the test was made, and who gave no history of acute infection during the past three months.

One strain of hemolytic streptococcus of the beta type has been employed in all the tests. The strain was derived from the throat of a patient suffering from rheumatic fever. It was highly active, and its fibrinolytic activity did not seem to change during the 6 months required for the tests. Fresh 18-hour old cultures were always used.

Numerous trials with varying amounts of the different agents have been made. At first we tested the ability of a serum, from individuals whose plasma was resistant, to render normal plasma clot insusceptible. Small amounts of fibrinolytic culture were used, and the method was in many respects similar to the antistreptolysin titration of Todd (7). This method proved unsuitable, however, as the serum from resistant individuals conferred only a small amount of antifibrinolytic property upon normal plasma clots. We have found it more convenient to put up a series of dilutions of the active culture and to test their lytic effect upon a constant amount of plasma. In this way we were able to determine the smallest dose of active culture capable of causing lysis of the plasma clot. Thus, a more accurate grading of the resistance is made possible, and it is an easy matter to follow variations in this resistance from time to time.

We found that the peptone used in the broth, in some way affects the clot formation. Parke Davis peptone in particular made the coagulation of plasma incomplete, while Cogit peptone had a less disturbing effect. Furthermore, when Parke Davis peptone was used, the coagulated plasma was rolled up into a compact mass, leaving the rest of the tube's contents completely fluid. Owing to this retraction of the coagulum, it was often difficult to see the difference between the positive and negative reactions. After some trial experiments, we found that the tests were read more easily when Ringer's solution was used instead of normal saline. Solid coagulation appeared rapidly, and even after 20 hours' incubation, the tubes could be inverted without affecting the solid clot which adhered to the bottom and the sides of the tube. In the tubes where dissolution occurred, all evidence of clotted plasma disappeared, and the contents

became completely fluid. In a few tubes containing a small amount of active culture the liquefaction might be somewhat incomplete, but in any case there was a great difference between these tubes and the tubes in which the contents were solid and resistant. Thus, there was a distinct difference between the positive and the negative reactions. With this procedure we were able to use Parke Davis peptone throughout the work.

The technical procedures have in all other respects been those recommended by Tillet and Garner (1). We have used oxalated plasma, clotted with calcium chloride. The amount of anticoagulant, potassium oxalate, was 0.02 gram to 10 cc. blood. In the tests, 0.2 cc. plasma was used, and the reactions were always carried out within 3 hours after withdrawal of blood. Of a 0.25 per cent solution of  $\text{CaCl}_2$  in 0.85 per cent salt solution 0.25 cc. was used as coagulant.

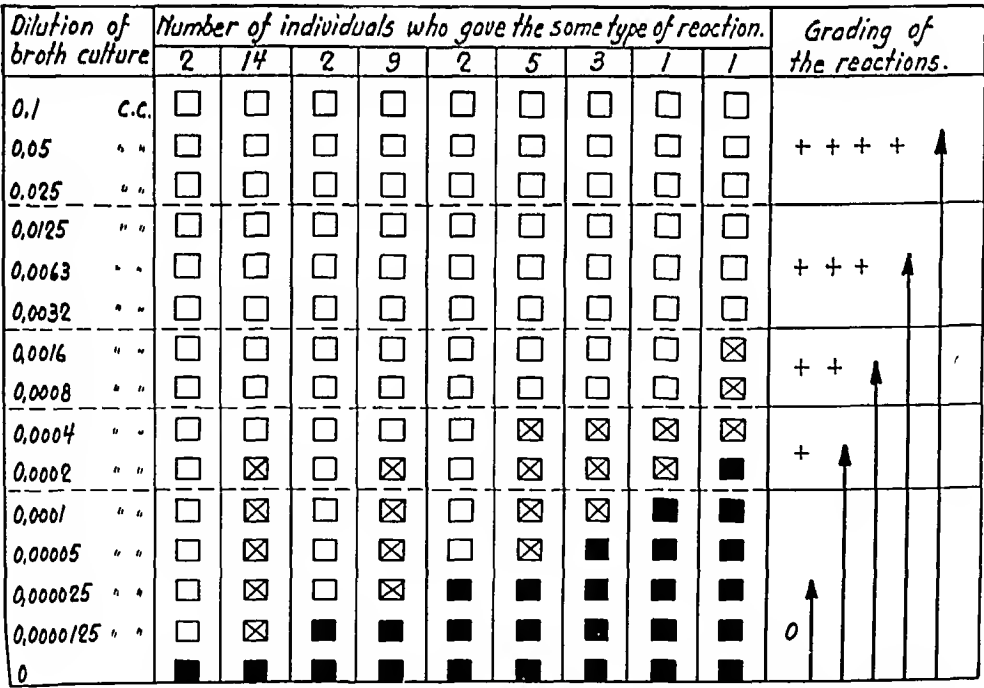
*Description of test.* Amounts of an 18-hour culture ranging from 0.1 cc. to 0.0000125 cc. respectively, were mixed with 0.5 cc. of Ringer's solution (see Figure 1). To each tube was added 0.8 cc. Ringer's solution, and then 0.2 cc. plasma. The coagulant, 0.25 cc. of the  $\text{CaCl}_2$  solution, was then added and well mixed. The tubes were incubated at 37° C.

A clot formed within a few minutes, and the liquefaction of a susceptible plasma clot started after ½ to 1 hour in the tubes containing the highest concentrations of active culture. All tests were read after 4 and after 20 hours of incubation. The results after 20 hours of

incubation proved to be the most reliable, and in this paper only the 20-hour results are given.

Normal controls

Thirty-nine "normal" subjects were tested. The results are shown in Figure 1, those from individuals yielding the same type of reaction being assembled in one column. In 16 of the normal subjects only the control tubes were left unaffected by the lytic principle. In two of these, the contents of all tubes containing active culture were completely liquefied. In the remaining 14, complete dissolution did not occur in a certain number of tubes (varying from 2 to 5), which contained the smallest amount of culture. Eleven subjects showed no dissolution in the tube containing the smallest amount of active culture (0.0000125 cc.). In two of these subjects the plasma was completely liquefied in all other tubes, while the remaining 9 subjects gave incomplete liquefaction in a varying number of the tubes (from 2 to 4) containing the smaller amounts of active culture. Seven individuals were resistant to the doses 0.0000125 cc. and 0.000025 cc. The



Each column represents a varying number of series of dilutions. The varying numbers are indicated at the head of each column.

■ : Solid after 20 hrs. incubation.  
⊠ : Not completely liquefied after 20 hrs. incubation.  
□ : Completely liquefied after 20 hrs. incubation.

FIG. 1. RESISTANCE TO FIBRINOLYTIC ACTIVITY IN NORMAL INDIVIDUALS.

plasma clots from the majority of the cases (34) were thus very susceptible; those from 5 were somewhat more resistant. Four of the latter patients could not be said to have had any streptococcal infections. The clot from the fifth subject, whose reaction is given last in the chart, was completely resistant up to the dose 0.0002 cc. He was more thoroughly examined, and admitted that his tonsils had been removed one and a half months previously, on account of repeated attacks of sore throat. This was probably the reason for the high resistance of his clot.

The reactions are graded as follows (Figure 1). In the patients whose plasma clot was resistant to all dilutions of active streptococcal culture broth up to the dose 0.025 cc. or higher, the designation is +++++. Resistance only up to 0.0125 cc., 0.0063 cc., or 0.0032 cc. is termed +++; up to 0.0016 cc. or 0.0008 cc. ++; and resistance only up to 0.0004 cc. or 0.0002 cc. is designated +. The reaction is regarded as negative or normal when the clot remains intact only in the presence of 0.0001 cc. or less of the broth culture of streptococcus used. The end point was read from the tubes in which the contents were completely solid after 20 hours of incubation, and not from those in which incomplete

liquefaction took place. There was thus only one of the 39 normal subjects who gave a weak positive antifibrinolysin reaction. The value of this rough grading is naturally restricted; for this reason we have, to a great extent, reproduced charts demonstrating the reactions obtained with plasma from the various patients.

### *Acute polyarthritis*

Plasma clots from 7 patients with acute polyarthritis were examined. All of the patients had had sore throat or more definite angina 1 to 2 weeks before the joint symptoms appeared. The antifibrinolysin reactions of the plasma from these patients are given in Figures 2, 3 and 4.

*Patient Number 1, E. J.* (Figure 2), 31-year old woman, went through a severe attack. Several joints were affected, but no carditis developed. On the 13th and 29th day after the onset of the first joint symptoms, her plasma clot was completely resistant. Following improvement, her plasma became more susceptible. She was discharged without any symptoms, and with a completely normal fibrinolysin reaction.

*Patient Number 2, P. R.* (Figure 2), 18-year old boy, had a severe attack. Several joints were swollen and tender. He had a mild carditis, with prolonged conduction time and deviation of the ST line which disappeared during the course of the disease. Thirty and 44 days after the onset of the joint symptoms the anti-

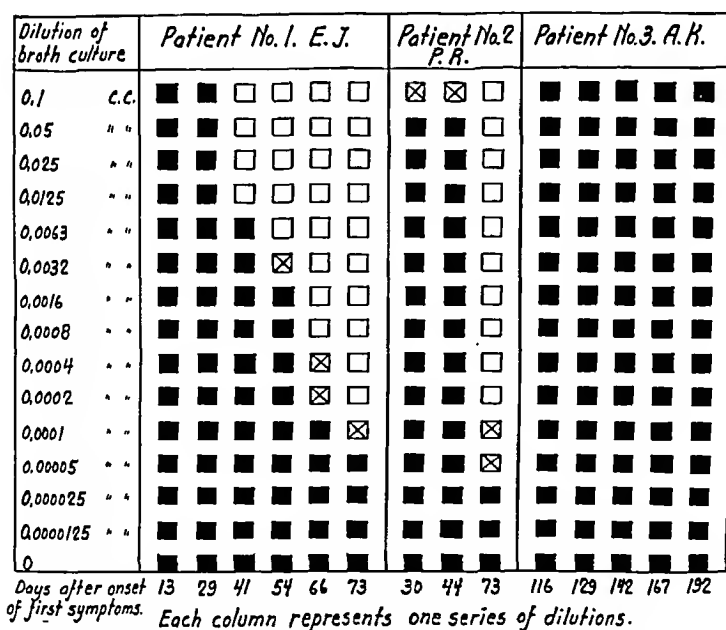


FIG. 2. RESISTANCE TO FIBRINOLYTIC ACTIVITY IN RHEUMATIC FEVER.



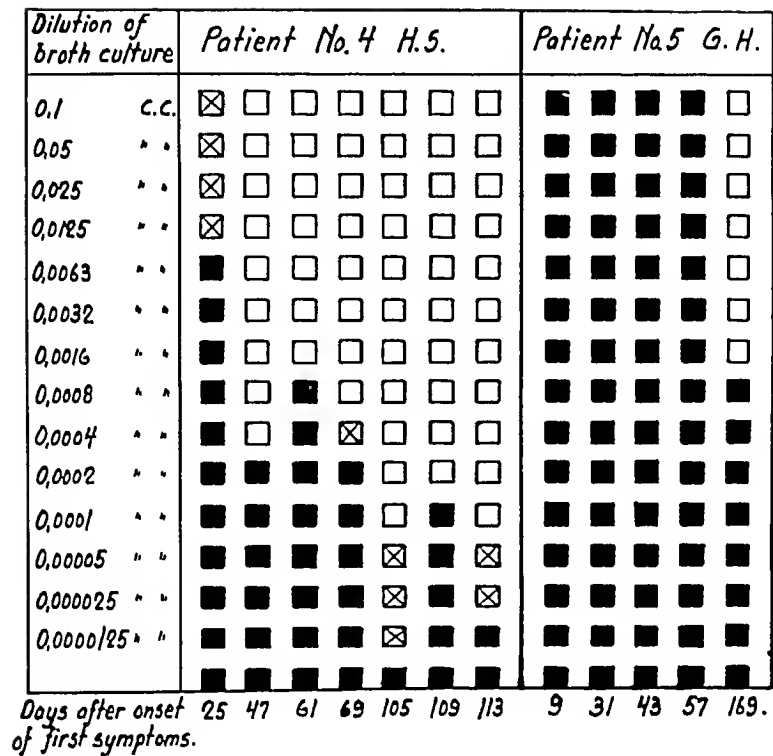


FIG. 3. RESISTANCE TO FIBRINOLYTIC ACTIVITY IN RHEUMATIC FEVER.

fibrinolysin reaction was completely positive. After 73 days his plasma clot was susceptible, and he was discharged without any symptoms.

*Patient Number 3, A. K.* (Figure 3), 12-year old girl, had a very severe attack with migrating polyarthrititis, and severe continuous carditis. She died after half a year's illness. The plasma clot from this patient was completely resistant during the time she was under the author's observation. The last test was performed 14 days before death.

*Patient Number 4, H. S.* (Figure 3), 36-year old man, had a history of rheumatic disease at the age of 9, with three recrudescences. The present was his fifth attack, and of average severity with a mild carditis; he had prolonged conduction time which became normal during the course of the disease. On the 25th day of disease his plasma clot was resistant though not completely so. The reaction was weaker on the 47th day. The plasma was, however, more resistant after 61 days. This was probably due to a mild recrudescence following the extraction of several infected teeth. After this, the reaction became weaker, and on the 105th day the clot was normally susceptible. Tonsillectomy was carried out and three days afterwards (on the 109th day) there was a slight rise in resistance of the plasma clot to fibrinolysis. Correspondingly he had a mild recrudescence of his disease of short duration.

*Patient Number 5, G. H.* (Figure 3), 25-year old man, experienced a typical attack of rheumatic fever at the age of 12 and developed heart failure. He was admitted with a history of insidious onset of the joint symptoms, and signs of severe continuous myocarditis

and endocarditis. He stayed in the hospital from the 9th to the 57th day of disease, and during this time the antifibrinolysin reaction was completely positive. After 5 months (169th day) his plasma clot was less resistant. During the time he had stayed at home his improvement had been steady and marked.

*Patient Number 6, A. T.* (Figure 4), 34-year old woman, was admitted with a severe acute polyarthrititis, the course of which proved to be insidious. No carditis was found. On the 4th and 8th day of the disease her plasma clot was somewhat more resistant than in normal subjects. However, the reaction was distinctly positive. The infection of the throat appeared 12 days before the first test was performed.

*Patient Number 7, A. R.* (Figure 4), 25-year old man, had a very mild and vague rheumatic attack of short duration without carditis. During the course of disease the antifibrinolysin reaction was completely normal. In this patient the first reaction was carried out 20 days after the manifestation of the angina.

In patients Numbers 1, 2, 4, and 5 the reactions were completely or almost completely positive about 4 weeks after the onset of the first joint symptoms. At this time the plasma clot of patient Number 6 had acquired normal susceptibility, whereas the reaction at an earlier stage of the disease was weakly positive. After 4 weeks, the polyarthrititis in patient Number 6 was distinctly active. Her condition was, however, better, and

Dilution of broth culture		Patient No. 6. A.T.				Patient No. 7. A.R.			
0.1	c.c.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.05	"	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.025	"	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0125	"	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0063	"	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0032	"	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0016	"	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0008	"	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
0.0004	"	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
0.0002	"	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
0.0001	"	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
0.00005	"	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
0.000025	"	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
0.0000125	"	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
0		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Days after onset of first symptoms.		4	8	28	37	11	20	28	49

FIG. 4. RESISTANCE TO FIBRINOLYTIC ACTIVITY IN RHEUMATIC FEVER.

correspondingly the weakly positive reaction disappeared.

In summary, it appears that extremely high resistance of plasma clots is associated with intense rheumatic activity. Thus, the clots from the two patients, Numbers 3 and 5, with the most severe disease, were most resistant to fibrinolysis. When the rheumatic manifestations disappeared, the antifibrinolysin reaction of the plasma returned to a normal level.

Throat cultures were studied from patients Numbers 1, 3, 4, 5, 6, and 7. In patient Number 1, no hemolytic strain was found. In patients Numbers 3, 4, and 5, on the other hand, hemolytic strains of streptococci were recovered from the throats, and these strains were actively fibrinolytic. In the last two patients, Numbers 6 and 7, no fibrinolytic strains were found, in spite of the fact that the tonsils were swabbed and cultured on blood agar plates six times and that a culture was made from the interior of the tonsils after their removal. From patient Number 6 we recovered no hemolytic strains, and the hemolytic strains recovered from patient Number 7 possessed no fibrinolytic activity. It is possible that these observations may be helpful in explaining the variations in reactions. It may thus be as-

sumed that the patients who harbor in their throats the strains with the most extreme fibrinolytic activity will give the most definite immune response and the most active disease picture. Our data are not sufficiently extensive, however, to justify an opinion on this point.

### *Chronic infective arthritis*

Twenty-four cases were studied. Five of the patients were children, probably belonging to the group of Still's disease. The other 19 were adults, and according to the clinical picture, they must be characterized as rheumatoid (atrophic) arthritis. Several of the patients had suffered from their disease for one year or longer, and all of them had active symptoms at the time the tests were performed. Roentgen examinations of the affected joints showed calcareous atrophy to a varying degree. In the more advanced cases, slight deformation of the joint surfaces had developed with formation of osteophytes. Seven of the patients were examined several times during the course of the disease (Figures 5 and 6).

*Patient Number 8, H. A.* (Figure 5), 31-year old man, for one year had had rheumatoid arthritis. Several joints were affected; no carditis was noted. He was febrile with a sedimentation rate of the red blood corpuscles of about 120 mm. after 1 hour. On March 1st and April 4th the antifibrinolysin reaction was positive, whereas the clot was normally susceptible on May 28th. During this time he had been treated with streptococcal vaccine, with no change in symptoms.

*Patient Number 9, G. N.* (Figure 5), 45-year old woman, was admitted with a story of polyarthritis of varying intensity for 6 months. Mainly the hips were affected. On arrival she was febrile, sedimentation rate 35 mm., no carditis. The first test on the plasma of this patient was positive. At the time of the last test the disease was less active, and her clot had normal susceptibility.

*Patient Number 10, E. R.* (Figure 5), 51-year old man, during 15 years was known to have had several attacks of fever and migrating polyarthritis. In the hospital he was subfebrile with a sedimentation rate of about 50 mm. He had severe carditis (heart block). The two tests carried out with the blood from this patient gave positive reactions. The disease was apparently less active at the time of the last test, and, correspondingly, the plasma clot was more susceptible.

*Patient Number 11, K. M.* (Figure 5), 33-year old woman, for two years had had joint symptoms of varying intensity. She was subfebrile, had no carditis, and the sedimentation rate on admission was 110 mm. It became normal during the time she stayed in the hos-

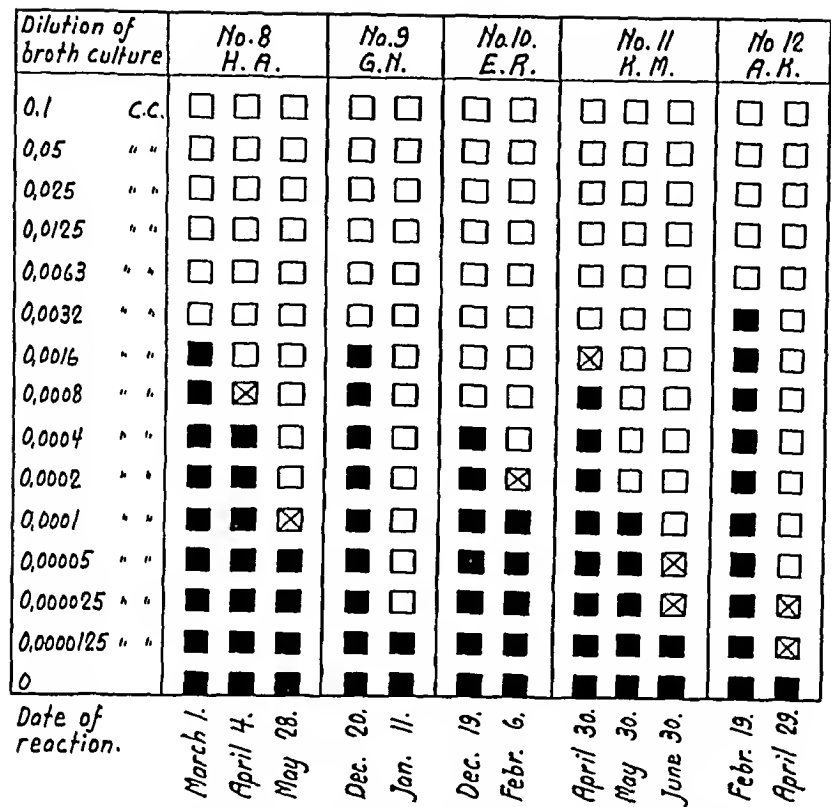


FIG. 5. RESISTANCE TO FIBRINOLYTIC ACTIVITY IN CHRONIC ARTHRITIS.

pital, and the joint symptoms nearly disappeared. The antifibrinolysin reaction was positive at the time of the most active symptoms, and the susceptibility of her plasma clot became normal following improvement.

Patient Number 12, A. K. (Figure 5), 50-year old woman, for several years had had rheumatic manifestations. On arrival, she was febrile, and her sedimentation rate was 45 mm. The electrocardiogram showed prolonged conduction time. At the time of the first test the reaction was positive, whereas her plasma clot showed a normal susceptibility two months afterward, when the symptoms of disease were less active.

Patient Number 13, B. S. (Figure 6), 26-year old woman, was admitted with a history of polyarthritis for one year. On arrival, she was febrile, with a sedimentation rate of 88 mm. She had several infected teeth and severe carditis (heart block). The three tests which were carried out on this patient showed normal susceptibility, in spite of the fact that the joint symptoms seemed to be active, especially at the time of the first test (January 6th).

Patient Number 14, O. K. (Figure 6), 26-year old woman, during two years had had symptoms of chronic arthritis of varying intensity. She was admitted with an involvement of several joints; afebrile; sedimentation rate 43 mm.; no carditis. Three tests were carried out, but the plasma clot was always susceptible.

In the other cases of polyarthritis, tests were performed only once during the course of the disease. The reactions given by the clots of some

of these patients are shown in Figure 6. Among these cases were patients with positive and patients with negative reactions. Cases Numbers 15 and 16 had very serious and intense manifestations, and the reactions were positive, whereas the disease picture had a milder and more indistinct character in the two others in which the reactions were negative (Numbers 17 and 18).

In some of our patients there was a distinct relationship between the antifibrinolysin reaction and the clinical symptoms; and the disappearance of resistance to fibrinolytic activity coincided with the improvement. These parallel changes in the reactions and the activity of the rheumatic manifestations occurred in patients Numbers 9, 10, 11, and 12. In patient Number 8, on the other hand, the resistance disappeared without improvement in the clinical symptoms. The reaction can, however, be completely negative during a period of several months in rheumatoid arthritis. This is exemplified in the two patients, Numbers 13 and 14, in which the activity of the disease manifestations may, of course, have been more marked at an earlier date.

The results obtained from plasma of patients with chronic arthritis are summarized in Table I.

Dilution of broth culture		No. 13 B.S.	No. 14 O.H.	No. 15 O.S.	No. 16 J.H.	No. 17 H.S.	No. 18 A.O.
0,1	C.C.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0,05	" "	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0,025	" "	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0,0125	" "	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0,0063	" "	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0,0032	" "	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0,0016	" "	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0,0008	" "	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0,0004	" "	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0,0002	" "	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0,0001	" "	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0,00005	" "	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
0,000025	" "	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
0,0000125	" "	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
0		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Date of reaction.		Jan. 9.	March 26.	May 7.	Jan. 28.	Febr. 28.	May 7.

FIG. 6. RESISTANCE TO FIBRINOLYTIC ACTIVITY IN CHRONIC ARTHRITIS.

TABLE 1  
*Antifibrinolysin reactions in chronic infective arthritis*

Number of cases	Type of reaction				
	++++	+++	++	+	0
19 adults.....	0	2	4	4	9
5 children.....	0	0	0	0	5

Five of the patients were children, 3 to 15 years old, and the disease picture in them was in most respects similar to that now known as Still's disease. They had had fever, and had had polyarthritis for several months or years. They had active symptoms at the time the blood was collected, but apparently some of them were improving. The plasma clot of these 5 children was susceptible, so that the reactions must be characterized as negative.

Of the 19 adults, 9 had negative and 10 positive reactions. Most of the reactions were weak. Among the 9 patients who gave negative reactions, only 2 (Numbers 13 and 14) were tested several times, and some of the patients who gave a positive reaction had a normal susceptibility later on in the disease. There is, therefore, reason to believe that some of the 9 patients with

the negative reactions would have shown a rise in resistance if we had been able to examine them at an earlier and probably more active stage of the disease.

#### *Bacterial endocarditis*

In this group specimens of plasma from 5 patients were tested. The disease picture corresponded in all cases to what now is known as "subacute bacterial endocarditis," or "endocarditis lenta." Among these, three had positive reactions, while the others showed normal resistance. A hemolytic fecal streptococcus was isolated from the blood of the first patient, E. H. (Figure 7). This strain did not, however, possess any fibrinolytic activity. In the 4 other patients, the disease was caused by a "*Streptococcus viridans*." Only two of the patients, namely F. M. and W. N., were known to have had rheumatic fever. It is at all events certain, that the two patients, E. H. and M. T., with the highest resistance, had no history of previous rheumatic attacks which could account for the positive reactions. It must, therefore, be assumed that the streptococcus found in the blood cultures had caused the rise in antifibrinolytic property of the

Dilution of broth culture	E. H.	M. T.	F. M.	W. H.	L. B.
0.1 c.c.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.05 " "	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.025 " "	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0125 " "	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0063 " "	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0032 " "	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0016 " "	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0008 " "	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0004 " "	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0002 " "	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
0.0001 " "	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
0.00005 " "	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
0.000025 " "	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
0.0000125 " "	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
0	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

FIG. 7. RESISTANCE TO FIBRINOLYTIC ACTIVITY IN BACTERIAL ENDOCARDITIS.

plasma clot. In the patient E. H. the fecal streptococcus, which in itself lacked fibrinolytic ability, had probably given rise to a distinct antifibrinolytic reaction. The same immune response also occurred in the patient M. T., in whom green streptococcus was the etiologic agent. This is of particular interest, because Tillett and Garner (1) have found that the strains belonging to the group of nonhemolytic streptococci never possess fibrinolytic ability.

#### DISCUSSION

The condition in the two last mentioned cases in the group of bacterial endocarditis seems to indicate that a rise in the resistance of blood to the fibrinolytic principle is not exclusively caused by the hemolytic and fibrinolytic streptococci of the beta type. We have previously shown (6) that other groups of organisms can induce a similar elevation of the resistance; for example, the plasma clots from patients suffering from pneumonia due to pneumococci were insusceptible. The antifibrinolysin reaction is therefore not specific. As a rule, however, the highest resistance seems to develop in patients suffering from hemolytic streptococcal infections. In rheumatic fever we have found that the most marked elevation of resistance to fibrinolytic activity occurs in the se-

vere cases, and that there is a close relationship between the activity of the rheumatic process and the antifibrinolysin reaction. These investigations thus support the theories of Coburn, inasmuch as Coburn and Pauli (8) have maintained that infection with toxin-producing strains of hemolytic streptococci in some way initiates the peculiar rheumatic process in susceptible subjects. The activity of the rheumatic process seems to depend upon the immune response of the rheumatic patients. The special mode, or mechanism of action of the streptococci is, however, not clear. Therefore, the question arises as to the rôle of the properties of the infecting organisms. Is it possible that the strains of streptococci having a low fibrinolytic ability, or none, are only to be found in cases with mild and vague manifestations? Patient Number 8 supports this probability, but further investigations are necessary.

It is very difficult to evaluate the results obtained in the cases of chronic infective arthritis. The degree of reaction has in 4 of the patients (Numbers 9, 10, 11, and 12) varied according to the intensity of the symptoms, and the plasma clots of 50 per cent of our patients suffering from rheumatoid arthritis showed increased resistance. The number of "positive" reactions exceeds that which Blair and Hallman (9) obtained when testing the streptolysin titer of similar patients. We consider it doubtful whether our results justify the conclusion that hemolytic streptococci are responsible for the immune response demonstrated, or that there is a significant association between these organisms and chronic infectious arthritis. This is a possibility, but our demonstration of a similar immune response in bacterial endocarditis indicates that other microbes in addition to hemolytic streptococci can cause a rise in antifibrinolytic properties of plasma clots.

At this point in our investigations we can only state that some of the patients suffering from rheumatoid arthritis give positive antifibrinolysin reactions in the active state of the disease, while others do not. To what extent the number of patients with a negative reaction can be reduced by examinations carried out at an earlier stage of the disease remains to be seen. It would probably prove helpful in evaluating the etiologic rôle of different organisms to examine at the same

time for the presence of agglutinins and precipitins for various streptococci and to perform antistreptolysin and antifibrinolysin tests. Great care should be taken to carry out the tests at an early stage of the disease, and to follow the reactions for a considerable period of time.

#### CONCLUSIONS

1. The majority of patients with rheumatic fever revealed a rise in resistance of their plasma to the fibrinolytic principle of the hemolytic streptococcus.

2. The strength of the reaction seemed to vary according to the severity of the symptoms of the disease.

3. The susceptibility of the plasma clot returned to normal when the patients improved.

4. The plasma clots of 50 per cent of the patients suffering from chronic infective arthritis showed slight elevation of resistance to fibrinolysis. Clots from five children with Still's disease gave negative reactions.

5. In patients suffering from chronic infective arthritis the antifibrinolysin reaction may change with the activity of the disease.

6. The plasma clot of patients with bacterial endocarditis caused by fecal streptococci and green streptococci may be resistant to the fibrinolytic principle of hemolytic streptococci of the beta type.

7. The antifibrinolysin reaction is, therefore, not specific, and does not decisively prove that an infection with hemolytic streptococci of the beta type has been experienced.

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# STUDIES OF HEMOLYTIC STREPTOCOCCAL INFECTION. III. THE CHARACTERISTICS OF THE HEMOLYTIC STREPTOCOCCI ISOLATED FROM PATIENTS WITH ERYSIPELAS<sup>1</sup>

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It is a matter of common experience that initial attacks or recrudescences of acute rheumatic fever frequently follow a hemolytic streptococcal infection of the throat such as acute follicular tonsillitis or scarlet fever. In the experience of Coburn and Pauli (1), recrudescences are determined by: (1) infections with a highly effective agent; (2) the disease pattern, peculiar to each rheumatic subject; (3) the intensity of the immune response of the patient as indicated by a rise in the anti-streptolysin titer of the blood.

During our studies of erysipelas (2, 3), we did not observe a single case of rheumatic fever following the infection, and the records of 1400 cases of erysipelas that were reviewed did not reveal any cases. Other studies fail to mention erysipelas as a preceding infection in rheumatic fever, whereas it is common knowledge that hemolytic streptococcal infections of the throat frequently precede attacks. Why this should be the case is not clear; but inasmuch as Coburn and Pauli have emphasized the importance of certain characteristics of the effective organisms in the determination of recrudescences of rheumatic fever, we have studied the hemolytic streptococci from cases of erysipelas to see whether or not they differed in any respect from those isolated from rheumatic subjects.

## METHODS OF STUDY

In the cases of facial erysipelas, the organisms were isolated either from the lesion or the nose. A small amount of sterile salt solution was injected into the edge of the lesion and, after waiting for several minutes, material was then aspirated and placed in blood broth. Twenty-two strains were isolated and studied, according to the following standard methods: sugar fermentation

with one per cent lactose, mannite, salicin, trehalose, and sorbital, final pH in dextrose broth, the reduction of methylene blue, the hydrolysis of sodium hippurate, hemolysis on blood agar plates (4); the type of colony, on surface plates of neopeptone agar (5); the erythrogenic toxin production, the serological classification,<sup>2</sup> and the fibrinolysin production. Toxin-antitoxin neutralization tests were carried out when the presence of toxin was found. These results were correlated with the serological reactions that we observed in the patients.

From Table I it is seen that the final pH of all strains falls between 4.8 and 5.2. They all fermented trehalose but none fermented sorbital; none of the strains reduced methylene blue but 4 strains showed slight hydrolysis of sodium hippurate. These observations indicate that the strains were of the human type. This was confirmed by serological examinations, using the precipitin test with rabbit antiserum, as described by Lancefield (6). All of the strains fell into Group A or those of the human type.

Sugar fermentation tests with lactose, mannite, and salicin showed that, in accordance with the classification of Holman (7), there were 19 strains of *Streptococcus pyogenes*, 3 strains of *Streptococcus infrequens* and one strain of Hemolytic II in the group.

The appearance of the colonies on neopeptone blood agar plates was studied with a hand lens and with the low power of the microscope, using reflected light. Two types of colonies were observed. The one which was predominant had a smooth, shiny, convex surface; the other type of colony showed a depression in the center which

<sup>1</sup> This investigation was aided, in part, by a grant from the Milton Fund and Clark Bequest.

<sup>2</sup> We are indebted to Dr. Rebecca Lancefield of the Rockefeller Institute for Medical Research for sending us known strains of Groups A, B, and C which we used for producing immune serum in rabbits for precipitin tests.



TABLE I  
*Summary of corysipelas organisms*

Patient	Lactose	Mannite	Salicin	Trehalose	Sorbitol	Final pH	Methylene blue	Sodium hippurate	Type of colony	Hemolysis	Fibrinolysin	Toxin	Group	Antistreptolysin	Antifibrinolysin	Toxin antitoxin	Maximum number of organisms killed by	
																	Patients	Controls
1*	+	-	+	+	-	4.9	-	±	M	B	++++	++++	A	1,000	++++	+	10 <sup>-1</sup>	10 <sup>-4</sup>
2	-	+	+	+	-	4.8	-	±	M	B	++++	++++	A	3,000	++++	0	10 <sup>-2</sup>	10 <sup>-6</sup>
3*	+	-	+	+	-	4.8	-	-	M	B	++	++++	A	500	++	0	10 <sup>-1</sup>	10 <sup>-7</sup>
4*	+	-	+	+	-	5.0	-	-	M	B	+++	++++	A	500	++++	0	10 <sup>-1</sup>	10 <sup>-4</sup>
5*	+	-	+	+	-	5.0	-	-	M	B	-	++++	A	1,000	++	0	10 <sup>-1</sup>	10 <sup>-6</sup>
6	+	-	+	+	-	5.2	-	-	F	B	++++	++++	A	500	++++	0	10 <sup>-1</sup>	10 <sup>-6</sup>
7	+	-	+	+	-	5.2	-	-	M	B	++++	++++	A	200	++++	0	10 <sup>-1</sup>	10 <sup>-6</sup>
8*	+	-	+	+	-	5.0	-	-	M	B	++++	++++	A	800	++++	0	10 <sup>-1</sup>	10 <sup>-1</sup>
9	+	-	+	+	-	5.0	-	-	M	B	++++	++++	A	800	++++	0	10 <sup>-1</sup>	10 <sup>-7</sup>
10*	+	+	+	+	-	4.8	-	-	M	B	++++	+	A	800	++++	0	10 <sup>-1</sup>	10 <sup>-6</sup>
11*	+	-	+	+	-	4.8	-	-	M	B	++++	+	A	100	++++	0	10 <sup>-2</sup>	10 <sup>-6</sup>
12	+	-	+	+	-	4.8	-	-	M	B	++++	+	A	500	++++	+	10 <sup>-2</sup>	10 <sup>-2</sup>
13	+	-	+	+	-	5.0	-	-	F	B	++++	+	A	1,000	++++	0	10 <sup>-1</sup>	10 <sup>-6</sup>
14	+	-	+	+	-	4.8	-	±	F	B	++	+	A	3,000	++	0	10 <sup>-1</sup>	10 <sup>-4</sup>
15*	+	+	+	+	-	4.8	-	-	M	B	++++	+	A	3,000	++++	0	10 <sup>-1</sup>	10 <sup>-4</sup>
16	+	-	+	+	-	5.2	-	-	F	B	++++	+	A	500	++++	0	10 <sup>-4</sup>	10 <sup>-6</sup>
17*	+	-	+	+	-	4.8	-	-	M	B	++	0	A	300	+	-	10 <sup>-1</sup>	10 <sup>-2</sup>
18*	+	-	+	+	-	4.8	-	±	M	B	++	0	A	1,000	++++	-	10 <sup>-1</sup>	10 <sup>-1</sup>
19	+	-	+	+	-	4.8	-	-	M	B	+	0	A	500	++++	-	10 <sup>-1</sup>	10 <sup>-4</sup>
20	+	-	+	+	-	4.8	-	±	M	B	++	0	A	3,000	++++	-	10 <sup>-1</sup>	10 <sup>-2</sup>
21*	+	-	+	+	-	4.8	-	-	M	B	++	0	A	1,000	++++	-	10 <sup>-1</sup>	10 <sup>-1</sup>
22	+	-	+	+	-	4.8	-	-	M	B	++	0	A	1,000	++++	-	10 <sup>-1</sup>	10 <sup>-1</sup>

\* Organism isolated from nose. Others isolated from the lesion.

gave it a concave appearance. The smooth shiny colonies correspond to the M or matt or mucoid forms, and the concave surface colonies correspond to the F, flocculent form of Ward and Lyons (5) or, possibly, the smooth convex form of Dawson and Olmstead (15). There were 19 strains with the M forms and 3 with the F forms. In our experience, 16 of the 19 strains grew diffusely in neopeptone broth, the other grew in a flocculent manner. Seven strains agglutinated spontaneously in plain broth, and only one strain agglutinated spontaneously in serum neopeptone.

#### *Production of skin toxin*

The toxin production of the twenty-two strains was studied by testing the capacity of each filtrate to produce an erythematous reaction in the skin of an individual who was Dick positive. The filtrate was produced by growing the organisms for 48 hours in .02 per cent dextrose-beef infusion broth and filtering it through candles. The skin of an individual who reacted positively to Dick toxin was used for testing. Dilutions of

filtrate 1:100 were injected intracutaneously. Control observations were carried out with broth and heated filtrate. All readings were made at the end of 24 hours and recorded as negative, weak, or strong. The strong reactions gave an erythematous papule greater than one centimeter in diameter; a weak reaction was an erythema of one centimeter or less; a negative reaction, no reaction at all. The results are listed in Table I.

After it was determined which strains produced toxin that was powerful enough in dilutions of 1:100 to cause a positive skin reaction, it was determined whether these toxins could be neutralized by scarlet fever antitoxin. For these tests, the toxin was mixed with the antitoxin. From preliminary titrations with Dick toxin in a susceptible individual, it was found that 0.1 cc. of a 1:100 dilution of toxic filtrate, which we made from a scarlet fever streptococcus (NY5), was neutralized by 0.1 cc. of a diluted antiserum of a potency that contained a neutralizing capacity of 20 S.T.D. of the standard toxin. We used, therefore, the same dilution of anti-scarlet fever serum and toxic filtrates in our neutralization

tests in studying the toxin filtrates of the erysipelas strains.

The results are presented in Table I. Nine of the strains were strong toxin producers, 7 were weak. Six strains produced no toxin. In all, then, 16 of the 22 strains, or 69 per cent, produced erythrogenic toxin, and the toxin from 14 of the 16 strains was neutralized by anti-scarlatinal antitoxin.

#### *Identification of the organisms with the Lancefield method*

Rabbits were injected with known strains of hemolytic streptococcus from Groups A, B, and C so that specific antisera were obtained that would react with the group-specific ("C" substance) antigen when the precipitin test was employed. Extracts of each strain were made and tested against the antisera for precipitins, using 0.4, 0.1, and 0.025 cc. of the extract with 0.2 cc. of antiserum. The reaction was read after 15 minutes and, finally, after standing 24 hours in the ice box. From these examinations, we found that all of the organisms were members of Group A and, therefore, of the human type. The only instance in which an organism belonging to a group other than A has been isolated from a case of erysipelas is the case reported by Griffith (14) in which the organism belonged in Group C.

#### *Fibrinolysin production*

The fibrinolytic activity of the different strains was tested by the method of Tillett and Garner (8). The plasma clot from an individual who had not experienced a hemolytic streptococcal infection for two years was used, and it had been determined on many occasions that some strains of hemolytic streptococcus were capable of digesting this clot within 15 minutes.

While the strains varied in their potency when a plasma clot derived from a single source was used, this was not surprising since the patients with erysipelas showed various degrees of resistance to fibrinolysin during the course of the disease. This may be due either to the difference in individual response or to the potency of the fibrinolysin produced. The degree of resistance to fibrinolysin in the different patients is revealed in Table I. It is safe to say that most of the

strains isolated from erysipelas produce fibrinolysin of high potency which is capable of exciting the production of antifibrinolytic substances in the host.

#### *Streptolysin*

We did not study the potency of the streptolysin production of the individual strains but, in view of the antistreptolysin titer of the serum that was observed in the patients infected by these strains, it is assumed that streptolysin was elaborated and there was a response on the part of the host to its production.

Ever since Fehleisen first described the streptococcus as the cause of erysipelas, there has been a lively discussion regarding the characteristics of the streptococci isolated to this particular form of infection. It has been maintained that the streptococci producing erysipelas form a special group of organisms which are distinct from those causing other forms of streptococcal infection. There is other evidence, both clinical and experimental that strains of hemolytic streptococci isolated from patients with erysipelas are made up of a number of serological types, and that they cannot be distinguished from scarlet fever strains by agglutination and absorption tests. Moreover, while it is clear that the different strains may be closely related to one another, they are also related antigenically to strains isolated from scarlet fever (9). In our experience, all strains have fallen into Group A of Lancefield's classification, although Griffith has described one strain from a case of erysipelas that belongs to Group C (Type 21, "Angel"). We have not studied the different specific types in this group of organisms.

There have been other observations which have attempted to discriminate erysipelas strains from scarlet fever and other strains of hemolytic streptococci on a basis of toxin production and toxin-antitoxin neutralization tests. The results of such studies have differed somewhat in the hands of various investigators and have resulted in a divergence of opinion regarding the specificity of organisms for particular types of infection. It has been shown by Wadsworth and Coffey (10), Hooker and Follensby (11), Trask and Blake (12), and Dick and Dick (13) that there are several different toxins elaborated by toxigenic

strains of hemolytic streptococci, and Hooker and Follensby have demonstrated two serologically distinct toxins from some single strains derived from scarlet fever. From the studies of Wadsworth and Coffey, in which it was demonstrated that toxigenic strains could be divided into several distinct groups, on a basis of toxin-antitoxin neutralization tests, there was no evidence of specificity of any of the groups for a particular type of infection. That is to say, there was no evidence from this work that streptococci derived from erysipelas were specific insofar as their toxin production was concerned.

In addition to this important observation, it was shown by these same investigators that antistreptococcal sera varied markedly in valency when toxin-antitoxin neutralization tests were carried out, and this valency was dependent upon the strains used in the production of antitoxic sera. One serum had a very broad valency and neutralized 77 per cent of the toxins. This serum was made from the Dochez NY5 strain. They found two other monovalent sera which were each effective against different groups of the remaining toxins studied which represented 21 per cent of the total and, finally, there were 5 strains among 314 whose toxins were only neutralized by combining two different sera. It is important, then, in studying toxin-antitoxin neutralization tests to use monovalent sera of broad valency before concluding that the toxin production from different strains is specific for particular types of infection.

From our experience, the toxins from 14 of 16 different strains were neutralized by antistreptococcal serum<sup>3</sup> which was made from Dochez NY5 strain. It cannot be concluded, therefore, that these tests demonstrated any specificity for these erysipelas strains insofar as toxin production was concerned.

The highest titer of the antistreptolysin in the blood of patients with erysipelas is recorded in Table I. There were wide variations but it was not uncommon to observe a vigorous response to streptolysin. The same may be said for the antifibrinolysin. Both of these tests indicate

that patients with erysipelas respond to the various antigenic components of the hemolytic streptococcus. This is also confirmed from a study of the bactericidal tests in the patients and controls, as previously reported by us (3) and as recorded in the table.

It is perhaps worthy of comment that the blood of normal controls killed only two of the toxin-producing strains in large numbers, whereas all of the non-erythrogenic toxin-producing strains except one were killed in large numbers. This would seem to indicate that normal controls possess fewer bacteriocidins against erythrogenic toxin-producing streptococci than against the non toxin-producing strains.

#### DISCUSSION

On the basis of the study of the above strains of hemolytic streptococci, it seems justifiable to say that these strains are no different from those isolated from other cases of human hemolytic streptococcal infections. Moreover, we have been unable to show that these strains differ in their biological characteristics and in their grouping from the strains that are isolated from cases of tonsillitis preceding rheumatic fever. It is our belief, then, that the reason for the uncommon occurrence of rheumatic fever following erysipelas cannot be attributed to the type of infecting organism, and certainly most patients with erysipelas have a vigorous immune response as indicated by a rise in the antistreptolysin titer.

It is possible that attacks of rheumatic fever were not observed in this group of cases on account of the small number studied, or it may have been due to the fact that all of our patients were adults belonging to an age period when attacks of rheumatic fever are less common. Hodges, of the Children's Hospital in Boston, informs us that rheumatic fever was not observed following erysipelas in a group of 75 children under 12 years of age. It would be desirable to obtain more information on the incidence of rheumatic fever following erysipelas in childhood, which is the period when rheumatic fever is seen most frequently. The question of age susceptibility is a most important factor in studying rheumatic fever. Until we know more about the various

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host factors in subjects with rheumatic fever, it would seem impossible to assess the relative importance of hemolytic streptococcal infections, and the intensity of the immune response in determining the inception or recrudescences of rheumatic fever.

#### SUMMARY AND CONCLUSIONS

A study of 22 strains of hemolytic streptococci derived from erysipelas yielded the following results.

1. All of the strains were of human origin (Group A), as determined by the method of Lancefield.

2. Sugar fermentations, the final pH in dextrose broth, the reduction of methylene blue, and the hydrolysis of sodium hippurate were in accord with the findings of other workers who have studied human virulent strains.

3. All of the strains produced beta type of hemolysis on blood agar plates.

4. All of the strains but 2 produced a strong fibrinolysin.

5. Sixteen of the 22 strains produced erythrogenic toxin, and the toxin from 14 of the 16 strains was neutralized by antiscarlatinal serum that was produced from the NY5 strain of hemolytic streptococcus.

6. The blood of normal controls contained a lower bactericidal titer for the toxin-producing strains than for the non-toxin-producing strains.

7. Patients infected with these various strains developed a good immune response as indicated by the antifibrinolysin, antistreptolysin, and the bactericidal content of their blood.

8. From the available evidence, the organisms derived from erysipelas do not seem to differ from those obtained from other human infections by the hemolytic streptococcus.

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# METABOLIC STUDIES ON CHRONIC ULCERATIVE COLITIS

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The object of this study was to obtain definite information on the metabolic status of patients who have severe chronic ulcerative disease of the colon, which is usually termed "chronic ulcerative colitis." To obtain this information we have made under controlled conditions extensive studies of the composition of the feces, urine, and food of three patients afflicted with this disease.

*Case 1* was a man, aged thirty-five years, who had an acute and advanced degree of the disease which involved the entire colon. This patient weighed 120 pounds (54.4 kgm.) and was 73 inches (185.4 cm.) in height. He had been in good health until six weeks prior to his admission to the hospital when he had begun to have diarrhea, cramps in the abdomen, and fever. He had lost 70 pounds (31.8 kgm.). Proctoscopic examination showed that the rectum, rectosigmoid, and sigmoid flexure were extensively ulcerated, scarred, and bleeding. Microscopic and bacteriological examinations of the feces revealed nothing of known importance. The value for the hemoglobin was 12.6 grams per 100 cc. of blood. Three observations were made in this case. The periods of these observations were four days, four days, and two days respectively.

*Case 2* was a woman, aged forty-three years, who had had chronic diarrhea for two years, and a recent exacerbation. This patient weighed 137 pounds (62.1 kgm.) and was 63 inches (160 cm.) in height. She had lost sixteen pounds (7.3 kgm.). The entire colon was involved in the ulcerating process. Proctoscopic examination revealed numerous ulcerations in the rectum and sigmoid flexure. Microscopic and bacteriological examinations revealed nothing of known significance in the feces. There was 11.7 grams of hemoglobin in each 100 cc. of blood. Two observations were made in this case. The periods of these observations were three days each.

*Case 3* was a man, aged twenty-five years, who weighed 110 pounds (49.9 kgm.) and was 67 inches (170.2 cm.) in height, who had had diarrhea for two years, at times with acute severe exacerbations. The rectum and sigmoid flexure were ulcerated, scarred, and bled easily. The entire colon was involved in the diseased process. Microscopic and bacteriological examinations revealed nothing of known etiological significance in the feces. The value for the hemoglobin was 10.7 grams per 100 cc.

of blood. The duration of the one period of observation in this case was six days.

## THE GENERAL PLAN OF STUDY

*Procedure for the intake.* Cases 1 and 3 received a balanced adequate diet. There was no variation in the meals from day to day. The diet consisted of meat, milk, cream, eggs, butter, rice, potato, fruits and vegetables. Three meals were given daily. Sodium chloride was added to the diet in weighed amounts. Distilled water was used for cooking and was given by mouth in measured amounts daily. The monotony of this unchanged diet was tolerated for as long as two weeks and in neither case did estimations have to be made for food unconsumed. An analysis of a twenty-four hour diet was made. Case 2 was unable to eat as great an amount of food as were Cases 1 and 3, and she could not be depended on for any regularity of consumption. Accordingly, she was allowed to select her diet, and the same amounts of food which she consumed daily were analyzed. Her food was of the same general nature as that which was fed to the other two patients.

*Analysis of foods.* Analyses for nitrogen by the Kjeldahl method, fat (16), and chloride were made on a ground wet suspension of the food. An aliquot was dried and ground for determination of total solids and the amount of water. From this ground preparation, an ash was made in platinum dishes, using a muffle furnace, the temperature of which was kept below 550° C. Quantitative determinations of sodium, potassium, calcium, magnesium, and phosphorus were made on a hydrochloric acid extract of this ash. The total caloric value and the carbohydrate content of the foods were estimated from the tables of Sherman (18).

The urine was preserved with thymol and kept on ice. Daily estimations of the amount of creatinine were made as an additional check on the ac-

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curacy of collections. Quantitative determinations of chloride, nitrogen, and inorganic phosphorus were made directly on the urine. An ash was prepared for the determination of sodium, potassium, calcium, and magnesium.

The feces were collected in periods corresponding to a known intake of food, which were determined by charcoal markers. In some instances, daily separation of the feces was made; in others, several days were allowed to elapse before separation. For accuracy and convenience, certain of the analyses were done on two-day mixtures of feces, while others were done on three-day, four-day, and six-day mixtures. From six to twenty-five rectal discharges during twenty-four hours produces a great mass of feces. The collection of the large quantity of feces varied according to the condition of the patient. Case 1 had an incontinent anus which necessitated special collecting arrangements. To accomplish this, a clean rubber pad was kept underneath the patient at all times. Dejecta were then washed from the pad into suitable containers with known amounts of distilled water. Case 2 was able to use a suitable enameled bedpan. The collection of feces of Case 3 was made by the use of a specially constructed commode which permitted a direct collection of the feces in glass containers.

*Blood.* Various chemical and hematological determinations were made on the blood of these patients; the results of these determinations will be considered later.

*Physical characteristics of the feces.* The

weight of the feces excreted daily by these patients was excessive (Table I). For example, the first patient excreted from 745 grams to 1019 grams (90 per cent water) per day during the observation. The total dry weight, which was greater than normal, however, in each period, varied from 84 grams to 101 grams. In Cases 1 and 2 the values for total weight of the feces excreted daily and the weight of the dry substance were greater than they were in Case 3. The colon of Case 1 was much more extensively diseased than were the colons of Cases 2 and 3. There was gross blood and some pus in each of the dejecta of Cases 1 and 2. No undigested food was seen at any time; each stool was examined grossly. Gross blood occasionally was noted in the feces of Case 3.

*Chemical analysis of food, urine and feces.* The results of the quantitative analysis of the food, urine, and the feces during the periods of observation will be considered together. In Table II the data have been recorded and a balance has been indicated. These results are expressed in average number of grams ingested or excreted daily. References to the methods used will be found in the tables.

*Excretion of organic material.* The presence of a daily fecal excretion of nitrogen far in excess of normal constitutes one of the most interesting findings of these observations. This loss of nitrogen from the body through the feces has not, however, resulted in a net loss of nitrogen or a considerably negative nitrogen balance in Case 1. During the two months that Case 1 was observed after this study, the course was one of slow improvement, gain in body weight and improvement in health. The mechanism for conservation of body protein is evidenced by the low values for the total nitrogen excreted in the urine daily.

It has been indicated that Case 2 was unable to consume a satisfactory quantity of protein. During Period 1, an average of 4.69 grams of nitrogen per day was ingested in contrast to the total daily consumption of 12.61 grams of nitrogen by Case 1. During Period 1, the total excretion of nitrogen in the urine was 4.03 grams daily in Case 2. Obviously, the patient was existing on a low nitrogen catabolism. Examination of the nitrogen partition products of the urine excreted

TABLE I  
*Physical properties of the feces*

	Case 1			Case 2		Case 3
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 1
Total weight of feces, grams* . . . . .	745	902	1019	489	383	363
Total dry substance, grams* . . . . .	84	91	101	72	53	30
Total water, grams* . . . . .	661	811	918	417	330	333
Percentage of water . . . . .	89	90	90	85	86	92
Percentage of dry feces . . . . .	11	10	10	15	14	8
Average number of daily dejecta . . . . .	22	24	21	21	17	6

\* Grams excreted daily.

TABLE II

*Results of analysis of food, urine, and feces in three cases of chronic ulcerative colitis, expressed in grams per day*

	Period 1					Period 2					Period 3				
	Intake	Output			Balance or difference	Intake	Output			Balance or difference	Intake	Output			Balance or difference
		Urine	Feces	Total *			Urine	Feces	Total			Urine	Feces	Total	
Case 1															
Nitrogen †...	12.61	4.36	7.06	11.42	+1.19	12.61	4.74	8.21	12.95	-0.34	12.61	4.24	9.30	13.54	-0.93
Fat †.....	150.3		8.4		+141.9	150.3		8.8		+141.5	150.3		11.2		+139.1
Sodium §...	2.72	0.61	1.72	2.33	+0.39	2.72	0.33	2.22	2.55	+0.17	2.72	0.07	2.4	2.47	+0.25
Potassium   ...	2.95	1.81	0.77	2.58	+0.37	2.95	1.85	0.93	2.78	+0.17	2.95	1.67	0.97	2.64	+0.31
Calcium   ...	1.10	0.168	1.28	1.45	-0.35	1.10	0.203	1.18	1.38	-0.28	1.10	0.155	1.26	1.42	-0.32
Magnesium   ...	0.224	0.074	0.157	0.231	-0.007	0.224	0.073	0.180	0.253	-0.029	0.224	0.091	0.232	0.323	-0.099
Chloride ¶...	4.59	2.24	1.96	4.20	+0.39	4.59	2.27	2.63	4.90	-0.31	4.59	1.12	2.85	3.97	+0.62
Phosphorus **	1.43	0.56	0.83	1.39	+0.04	1.43	0.58	0.88	1.46	-0.03	1.43	0.49	0.92	1.41	+0.02
Water ††.....	3360	1466	661	2127	+1233	4360	2347	811	3158	+1202	2860	630	918	1548	+1312
Case 2															
Nitrogen †...	4.69	4.03	7.42	11.45	-6.76	4.19	4.65	6.15	10.80	-6.61					
Fat †.....	55.8		9.1		+46.7	34.9		5.0		+29.9					
Sodium §...	0.803	0.027	1.03	1.06	-0.26	0.639	0.023	0.727	0.750	-0.11					
Potassium   ...	0.710	0.110	1.07	1.18	-0.47	0.620	0.074	0.947	1.02	-0.40					
Calcium   ...	0.154	0.017	0.225	0.242	-0.088	0.123	0.022	0.175	0.197	-0.074					
Magnesium   ...	0.063	0.012	0.072	0.084	-0.021	0.044	0.010	0.062	0.072	-0.028					
Chloride ¶...	1.36	0.47	1.28	1.75	-0.39	1.06	0.46	1.01	1.47	-0.41					
Phosphorus **	0.477	0.211	0.507	0.718	-0.241	0.302	0.289	0.427	0.716	-0.414					
Water ††.....	2241	1415	417	1832	+409	2258	1652	330	1982	+276					
Case 3															
Nitrogen †...	11.49	6.96	2.99	9.95	+1.54										
Fat †.....	90.4		5.6		+84.8										
Sodium §...	2.05	0.593	0.44	1.03	+1.02										
Potassium   ...	2.83	0.313	1.17	1.48	+1.35										
Calcium   ...	0.963	0.162	0.644	0.806	+0.157										
Magnesium   ...	0.208	0.041	0.126	0.167	+0.041										
Chloride ¶...	3.69	1.51	0.56	2.07	+1.62										
Phosphorus **	1.32	0.446	0.555	1.00	+0.32										
Water ††.....	3018	1264	333	1597	+1421										

\* In urine and feces.

† Determined by Kjeldahl's method.

‡ Determined by Saxon's modification (16) of Fowweather's method.

§ Determined by method of Butler and Tuthill (2).

|| Determined by method of Tisdall and Kramer (21).

¶ Chloride in urine was determined by Folin's (8) modification of the Voldhard method; chloride in food and feces was determined by method of Peters and Van Slyke (15).

\*\* Determined by method of Fiske and Subbarow (4).

†† Includes water in food.

in one day (Table III) reveals that the value for the urea nitrogen plus that for the ammonia nitrogen was reduced. The total value for the urea nitrogen and ammonia nitrogen amounted to only 60.8 per cent of the total nitrogen. The values for the creatinine, creatine, uric acid, and amino-acid nitrogen were not greatly increased. There was a measurable increase in the value for ammonia nitrogen. Gamble, Ross and Tisdall (10) have demonstrated in their studies on fasting children that such an increase in the value for ammonia nitrogen accompanies efforts to conserve the total base of the body. In Case 2, there was considerable loss of base through the intestinal tract,

which necessitated marked curtailment in the excretion of base in the urine and notably the excretion of sodium. The excretion of nitrogen in the feces reached a considerable magnitude (7.42 grams daily in Period 1 and 6.15 grams daily in Period 2). These amounts represent 64.8 per cent and 56.9 per cent respectively of the total output during Period 1 and Period 2. The total excretion of nitrogen in Period 1 was 6.76 grams more than was ingested. In Period 2, the amount of total nitrogen excreted was 6.61 grams more than was ingested. It is apparent that the large negative nitrogen balance resulted from the excessive amount of this substance in the feces, which

TABLE III

Average daily values for nitrogen partition products in the urine

Partition products	Case 2		Case 3	
	Grams	Per-centage of total nitro-gen	Grams	Per-centage of total nitro-gen
Total nitrogen*	3.26		7.0	
Urea nitrogen†	1.32	40.5	4.65	66.0
Ammonia nitrogen†	0.663	20.3	0.803	11.0
Creatinine nitrogen‡	0.232	7.1	0.378	5.4
Creatine nitrogen‡	0.157	4.8	0.079	1.1
Uric acid nitrogen§	0.151	4.6	0.212	3.0
Total amino acid nitrogen	0.192	5.9	0.222	3.2
Rest nitrogen	-0.545	16.7	+0.656	9.4

\* Determined by method of Kjeldahl.  
† Determined by the Van Slyke and Cullen modification (23) of Marshall's method.  
‡ Determined by method of Folin (5) (boiling with picric acid).  
§ Determined by the method of Folin and Wu (6); Folin and Marenzi (7).  
|| Determined by the Van Slyke (22) volumetric method.

was approximately five or six times that found in normal feces. In this case we have concrete evidence that a large portion, if not all, of the fecal nitrogen is contributed to the dejected mass by the body itself, presumably from the elimination of body tissues and blood in the colon, inasmuch as during both periods of observation the daily output of nitrogen in the feces exceeded the amount of nitrogen ingested.

In Case 3, there was a positive balance for nitrogen; 1.54 grams more nitrogen was ingested than was excreted. This net gain of nitrogen in the body occurred while 2.99 grams of nitrogen were excreted daily in the stool. This patient did not excrete as large an amount of nitrogen in the feces as did Cases 1 and 2. It has been previously noted that only occasionally was gross blood found in the stools in Case 3 and that the number of daily rectal discharges was less than in Cases 1 and 2. There was a reduction in the urinary excretion of nitrogen just as there was in Cases 1 and 2. The amount of nitrogen ingested daily was 11.49 grams and the amount excreted daily in the urine was 6.96 grams. This indicates that a positive balance was made possible by the reduction in the urinary excretion of nitrogen. To contrast these data with those of a normal adult who excretes in the urine as nonprotein nitrogen

TABLE IV

Average daily values for amount of fat in food and feces

Case	Period of observation	Fat in food *			Fat in feces *†				
		Grams	Neu-tral fat	Fatty acids	Grams	Neu-tral fat	Fatty acids	Per-centage of that ingested	Percentage of total dry substance of feces
1	1	150.3	per cent 05.6	per cent 4.4	8.4	per cent 42.8	per cent 57.2	5.6	10.0
	2	150.3	05.6	4.4	8.8	46.7	53.3	5.9	9.7
	3	150.3	05.6	4.4	11.2	47.0	53.0	7.5	11.1
2	1	55.8	97.1	2.3	9.1	26.0	73.1	16.3	12.7
	2	34.9	95.9	4.1	5.0	38.9	61.1	14.3	9.4
3	1	90.4	96.9	3.1	5.6	45.3	54.7	6.2	18.8

\* Determined by Saxon's modification (16) of Fowweather's method.  
† No soaps were present.

an amount equivalent to that which he ingests, suggests to us that this loss of nitrogen in chronic ulcerative colitis is not the result of unabsorbed food (1, 13, 17, 19) but is attributed to a loss of body tissue.

The amount of fat in feces and food have been separately recorded in Table IV. The largest amount of fat eliminated daily occurred in Case 1 in the third period. The amount eliminated represented only 7.5 per cent of the fat ingested. Case 2 consumed an average of 55.8 grams and 34.9 grams of fat in Periods 1 and 2, respectively. The fat eliminated during these periods represented 16.3 per cent and 14.3 per cent respectively of that ingested. Although the actual values for fat in the feces of Case 2 are not large, the small amount of fat ingested produces a calculation for an elimination that is out of proportion to the amount ingested. It is usually estimated that fecal fat amounts to 5 or 10 per cent of the fat ingested (14), although fecal fat is probably not, under normal conditions, derived from undigested and absorbed food. Hill and Bloor (11) have demonstrated that fecal fat is quite constant for a given individual and that it is dependent on the diet. Sperry (20) has demonstrated that the excretion of fat by dogs which have been deprived of fat in their diet remains constant. Fowweather (9) has determined that if the amount of fat in stools comprises more than 28 per cent by weight of the total dry substance the condition is abnormal. In none of these three cases did the fat in the feces

reach such a quantity. In Case 3 the quantity of fat in the feces amounted to 18.8 per cent of the total dry substance. This was the largest value obtained in any of these observations. In contrast to the fecal excretion of nitrogen, there was no evidence of an increased elimination of fat in the feces in any of these cases.

These three cases of chronic ulcerative colitis had a normal metabolism of fat and an abnormal metabolism of nitrogen. In Case 3 the excretion of nitrogen in the feces was only slightly increased. The absence of a markedly increased excretion of nitrogen in the feces in this case is in contrast to the fecal excretion of nitrogen in Cases 1 and 2. In the latter cases there was a normal metabolism of fat in the presence of a grossly abnormal nitrogen metabolism. There were two salient findings in Case 3: (1) there was a definite increase in the excretion of nitrogen in the feces, which occurred in the face of a net gain of nitrogen to the body, and (2) the losses of nitrogen from the body through the feces were not excessive. Apropos of this there were fewer bowel movements, than there were in Cases 1 and

2, and the feces did not contain much blood; these features indicate the mildness of the disease process in the colon. The fewer number of bowel movements in Case 3 tempts one to make a most obvious inference, namely, that in Cases 1 and 2 in which large amounts of nitrogen were found in the feces, the food has been rushed through the digestive tract and has not had an opportunity to be absorbed. However, the frequent defecations present in Cases 1 and 2, did not hinder the digestion and absorption of fat. The fact that these two patients normally absorbed fat gives the impression that the number of defecations in cases of ulcerative colitis does not have an important relation to the passage of food through the gastrointestinal tract but that the metabolic disturbances have a direct relation to the degree of disease process in the colon which permits the loss of blood and other body fluids and tissues.

The results of the analyses for the inorganic constituents of food, urine and feces have been recorded in Table II. The distribution of the daily output of inorganic constituents in the urine and feces has been recorded in Figure 1.

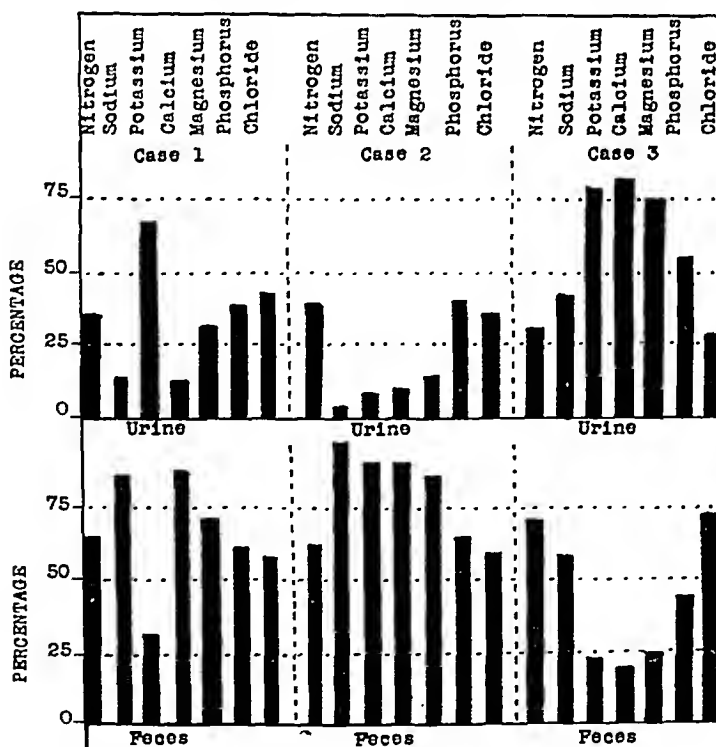


FIG. 1. PERCENTAGE DISTRIBUTION OF NITROGEN AND INORGANIC CONSTITUENTS IN URINE AND FECES

The amounts of sodium excreted daily in urine and feces were less than the amounts ingested in Cases 1 and 3. This apparently is a positive sodium balance. Excretion of sodium in the urine and feces in Case 2 exceeded the amount ingested.

The percentage distribution of sodium in the feces and urine is shown in Figure 1. It is apparent that the excretion of sodium in the feces greatly exceeds that in the urine. In Case 1, 73.8 per cent of a total of 2.33 grams were eliminated in the feces in Period 1. The output of sodium in the feces also was greater in each of the other two cases. However, the most marked example of the relative increase in the fecal excretion of sodium occurred in Case 2 (Figure 1). The urinary excretion of sodium was relatively low in all cases. Feces usually contain only 1 to 2 per cent of the total sodium excreted daily (14). In these cases, as much as 97.5 per cent of the total sodium eliminated per day was found in the feces. An inquiry into the cause of this increase in the excretion of sodium in the feces leads to the suspicion that it was the result of the large losses of intestinal fluids. The total amount of water lost daily varied from 918 grams to 330 grams and, in general, the more water eliminated, the more sodium was present in the feces.

In a previous paper by Welch, Wakefield, and Adams (25), it has been indicated that fluid that has a total base concentration of approximately 150 to 170 m.eq. per liter, 120 to 130 m.eq. of which are made up of sodium, is delivered to the colon to be absorbed. It was further indicated that losses from an ileac stoma caused a decrease in the excretion of sodium in the urine. This decrease in the urinary excretion of sodium demonstrates that there is a compensatory mechanism designed to maintain the total electrolyte of the body fluids near a constant value. The excretion of sodium in the feces of these patients is comparable to that observed in a case in which the patient has an ileac stoma. Intestinal fluid was delivered to the colon from the small intestine in quantities which could not be absorbed because of the diseased mucous membrane of the bowel. This fluid was therefore passed along and lost with the feces.

The distribution of total output of potassium

in the urine and feces is also abnormal because the total amount of daily feces is greater than normal. In Case 2, as much as 92.7 per cent of the total potassium eliminated in Period 2 occurred in the feces. Case 3 was in positive "balance." There was evidence of a marked potassium retention. We have been unable to determine whether or not the fecal excretion of potassium parallels the fecal solids. However, the excretion of sodium is proportional to that of water. The increase in the amount of fecal potassium in these cases would seem to be the result of augmentation of the feces by desquamated epithelium, tissue from the ulcerated wall of the colon, and blood.

The excretion of calcium in these cases was largely by the intestinal route; from 80 to 93 per cent of the total daily excretion of calcium was found in the feces in Cases 1 and 2. The calcium excreted in the urine was markedly decreased in all cases. It would seem that in Cases 1 and 2 more calcium than normal was lost through the gastro-intestinal tract and that the values for the blood calciums were also low. The excretion of magnesium and phosphorus was mainly through the feces.

Losses of chloride in the feces were substantial and paralleled to some extent the losses of sodium. The loss of sodium through the intestinal tract, however, was relatively greater than was the loss of chloride. This may be explained by the fact that the intestinal fluid contains relatively less fixed acid than it does fixed base. From 73.1 to 27 per cent of the total chloride was excreted by the intestinal route. In some of the periods, the chloride balance was negative.

The values obtained for serum sodium and the chloride in the plasma (Table V) indicate that the continuous loss of intestinal fluid resulted in a lowering of the concentration of these substances. Values for serum sodium were found to be consistently low (from 297 to 306 mgm. per 100 cc.). The values for the chloride were as low as 300 mgm. per 100 cc. of plasma on one occasion. The large losses of calcium in the feces also has resulted in low values for this mineral. The concentrations of the other inorganic substances in the blood were normal.

TABLE V  
Results of analysis of serum and plasma

Case	Period of observation	Blood serum					Blood plasma chloride
		Calcium *	Sodium †	Potassium ‡	Magnesium §	Phosphorus §	
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
2	1	8.3	305	15.8	2.2	3.1	310
	2	8.8	297	18.7	2.3	4.0	300
3	1	8.8	299	20.9	2.1	4.1	337
	2	9.3	306	19.3	2.1	4.3	347

\* Determined by method of Clark and Collip (3).

† Determined by method of Butler and Tuthill (2).

‡ Determined by method of Kramer and Tisdall (12).

§ Determined by method of Fiske and Subbarow (4).

|| Determined by method of Van Slyke (24).

#### COMMENT

The data obtained in the study of these three cases of chronic ulcerative colitis, which were of different degree of severity, has indicated that in general the metabolic disturbance took a definite direction. Considerable losses of important bodily substances have occurred in these cases through excretion in the feces. The elimination of nitrogen in the feces may reach considerable magnitude and the amount of nitrogen excreted daily would seem to be directly proportional to the severity of the ulcerative process in the colon rather than to the amount ingested in the food. In the presence of great losses of nitrogen in the feces there was a small urinary excretion of nitrogen in comparison to the intake of nitrogen in Cases 1 and 3. A decrease in the urinary excretion of nitrogen is seen in conditions of lowered nitrogen intake, starvation in the growing child, recovery from starvation, and during lactation. In many of these states there is a low value for the blood urea. A low value for the blood urea is a consistent observation in cases of severe chronic ulcerative colitis. In such cases, the concentration of urea in the blood varies from 8 mgm. to 20 mgm. per 100 cc. The metabolism of nitrogen in ulcerative colitis, with its evidence of diminished protein catabolism, would seem to be comparable to that observed in the lactating animal in which the source of the nitrogen excreted in the milk is body tissue. In cases of chronic ulcerative colitis, the nitrogen is lost in

exudates and in blood from the ulcerative surface of the colon. In these conditions, the first claim for protein absorbed is for synthesis of tissue as long as there is an adequate amount of fat and carbohydrate available for combustion.

The excretion of fat in the feces in these cases of ulcerative colitis was not in excess of that seen in normal subjects under the same conditions of intake. The fat of the food ingested by these subjects was digested and absorbed as well as in healthy individuals, as far as can be ascertained.

The elimination of water in the feces was considerable in Case 1, less in Case 2, and least in Case 3.

We feel that the rôle which the diarrhea plays in conditioning deficiency states in cases of chronic ulcerative colitis by virtue of decreasing the absorption of food through rapid transit or through changes in the character of the small intestine has been overestimated.

It would seem that the greatest need of the body of a subject with ulcerative colitis is for protein. It would also seem to be of advantage to supply in abundance those minerals lost in excess in the feces and depleted from the body, as evidenced by their lowered concentration in the blood serum.

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# STUDIES OF GONOCOCCAL INFECTION. I. A STUDY OF THE MODE OF DESTRUCTION OF THE GONOCOCCUS *IN VITRO*<sup>1</sup>

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At the present time, little is known concerning the development of local or general immunity in patients with gonococcal infections. It is universally recognized that local infections are notoriously chronic and that repeated infections of the same individual are common. The study of the immune mechanism in animals has not been very satisfactory since it is an extremely difficult matter to establish a gonococcal infection in ordinary laboratory animals. It did not seem unreasonable to suppose that more information could be obtained concerning the mechanism of immunity from a study of several serological reactions that appear during the course of the natural infection in man. We have studied, therefore, various antibodies in the blood of patients with different types of gonococcal infection, viz., the complement-fixing antibodies, agglutinins, precipitins, and the bactericidal power of defibrinated whole blood upon the gonococcus. We have found a study of the bactericidal power of whole blood most serviceable, and at this time we present a detailed discussion of its action, together with an analysis of the action of the circulating leukocytes and serum, upon the gonococcus. The results of this and the other tests upon a series of patients with gonococcal infections and normal controls are presented in the second paper.

## METHODS

The bactericidal action of whole defibrinated blood, serum, or plasma was determined by the method of Todd as used by us (1) in studying the hemolytic streptococcus. To 0.5 cc. of whole defibrinated blood, serum, or plasma, in small pyrex tubes, varying dilutions of an 18-hour suspension of gonococci in ascitic fluid broth were added. The number of organisms in each tube was determined by the plating of 1 cc. of the con-

tents of tubes  $10^{-6}$  and  $10^{-7}$ . The tubes were sealed in a gas-oxygen flame and then rotated, in a box, for 36 to 48 hours in an incubator at  $37.5^{\circ}$  C. The tubes were then opened and the contents were cultured to determine the presence of living organisms.

Thirty-six strains of gonococci isolated from active cases of gonorrhea have been studied in this manner. They were obtained from urethral and cervical exudates, synovial fluid, tendon sheaths, conjunctivae, and the circulating blood. The strains were grown on horse blood agar plates with a pH of 7.8 and sub-cultured every 3 days. The identity of the organisms as gonococci was proved by agglutination reactions with a polyvalent antigonococcal serum, and by sugar fermentation tests.

The action of the leukocytes upon the gonococcus was investigated in two ways. First, quantitative phagocytic studies were made on the blood of patients with gonococcal arthritis, and of normal controls. The procedure was to add 0.1 cc. of an 18-hour ascitic fluid broth culture of gonococci to 0.5 cc. of whole defibrinated blood in small pyrex tubes. After sealing the tubes, they were rotated in the incubator for 15 minutes to 4 hours. It was found that 15 minutes was the optimal time for maximal phagocytosis to take place. Smears were made and stained with Wright's stain. Fifty polymorphonuclear leukocytes were counted, and the number of cells containing organisms was noted. The degree of phagocytosis was compared with the bactericidal action of whole blood on the same suspension of organisms. The second method was to remove the plasma from whole defibrinated blood and wash the cells 4 times with 0.85 per cent salt solution. One-tenth of a cubic centimeter of a suspension of gonococci was added to 0.25 cc. of cells suspended in 0.25 cc. of salt solution in pyrex tubes. The tubes were sealed and rotated for 15 minutes, and then opened to determine the

<sup>1</sup> This investigation was aided, in part, by a grant from the Milton Fund and Clark Bequest.



presence of living organisms and the degree of phagocytosis.

### RESULTS

#### *Bactericidal action of whole defibrinated blood, serum and plasma*

The results of 33 observations on the bactericidal action of the whole defibrinated blood and of serum or plasma alone are presented in Table I. Thirteen different strains of gonococci were used. One specimen of blood was obtained from each of 13 patients with various types of gonococcal lesions and 2 normal controls. It is to be noted that equivalent amounts of serum had essentially the same killing power as whole, defibrinated blood. Tests were also done with plasma to determine whether it had the same killing power as serum or whole blood. An examination of

Wright's stained smears made from the blood in which there was growth of organisms revealed that most of the polymorphonuclear leukocytes contained gonococci. However, similar smears examined from the blood in the tubes in which there was no growth failed to show either extracellular or intracellular organisms. In short, it would appear that when the gonococcus was killed by the blood, it underwent complete lysis.

#### *Bactericidal action of whole defibrinated blood, plasma and heated plasma*

It has been indicated already that the killing power of whole defibrinated blood for the gonococcus is essentially the same as that of plasma or serum. If this bacteriolytic action on the gonococcus is due to the combined action of anti-

TABLE I  
*Comparison of bactericidal power of whole defibrinated blood, serum, and plasma \**  
(Each row represents a tenfold dilution of the gonococcus)

Observation number	Patient number	Strain number	Whole defibrinated blood							Serum							Plasma							Number of organisms
			10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	
1	1	1	+	+	0	0	0	0	0	+	+	+	0	0	0	0								10 <sup>-7</sup> =5
2	2	2	+	+	0	0	0	0	0	+	+	+	0	0	0	0								10 <sup>-6</sup> =6
3	3	3	+	+	0	0	0	0	0								0	0	0	0	0	0	0	10 <sup>-6</sup> =5
4	4	4	+	+	0	0	0	0	0								0	0	0	0	0	0	0	10 <sup>-7</sup> =9
5	5	5	+	+	+	0	0	0	0								+	+	+	0	0	0	0	10 <sup>-7</sup> =8
6	6	6	+	+	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-5</sup> =3
7		6	+	+	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-5</sup> =3
8		13	+	+	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-5</sup> =4
9	7	7	+	+	0	0	0	0	0	+	+	+	0	0	0	0								10 <sup>-4</sup> =20
10		8	+	+	0	0	0	0	0	+	+	+	0	0	0	0								10 <sup>-5</sup> =4
11		7	0	0	0	0	0	0	0	+	+	+	0	0	0	0								?
12	8	8	+	+	+	0	0	0	0	+	+	+	0	0	0	0								10 <sup>-6</sup> =4
13		6	0	0	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-5</sup> =3
14	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-7</sup> =12
15	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-6</sup> =3
16	11	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10 <sup>-6</sup> =2
17	12	12	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	10 <sup>-5</sup> =2
18		7	0	0	0	0	0	0	0	+	0	0	0	0	0	0								10 <sup>-5</sup> =2
19		13	0	0	0	0	0	0	0	+	0	0	0	0	0	0								10 <sup>-5</sup> =4
20	13	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-6</sup> =4
21		13	0	0	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-6</sup> =4
22		7	0	0	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-5</sup> =2
23		12	0	0	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-5</sup> =4
24		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-5</sup> =4
25	Control A	9	+	+	+	+	+	+	+								+	+	+	+	+	+	0	10 <sup>-7</sup> =3
26		8	+	+	+	+	+	+	+								+	+	+	+	+	+	0	10 <sup>-6</sup> =10
27		6	+	+	+	+	+	+	+								+	+	+	+	+	+	0	10 <sup>-7</sup> =3
28		13	+	+	+	+	+	+	+								+	+	+	+	+	+	0	10 <sup>-7</sup> =3
29		1	+	+	+	+	+	+	+								+	+	+	+	+	+	0	10 <sup>-6</sup> =8
30	Control B	2	+	+	+	+	+	+	+	+	+	+	0	0	0	0								10 <sup>-6</sup> =2
31		12	+	+	+	+	+	+	+	+	+	+	0	0	0	0								10 <sup>-6</sup> =10
32		7	0	0	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-6</sup> =2
33		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0								10 <sup>-7</sup> =5

\* + = growth.  
0 = no growth.

TABLE II

*Comparison of bactericidal power of whole defibrinated blood, unheated plasma, and heated plasma \**

Observation number	Patient number	Strain number	Whole blood							Unheated plasma							Heated plasma							Number of organisms
			10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	
1	5	5	+	+	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	0	0	10 <sup>-7</sup> 8
2	4	4	+	+	+	0	0	0	0	+	+	+	0	0	0	0	+	+	+	+	+	+	+	10 <sup>-7</sup> 9

\* + = growth.  
0 = no growth.

body and complement in serum or plasma, then heating either one to inactivate complement should result in reducing the killing power. Table II shows the results of heating plasma at 56° C. for 30 minutes in a water bath and comparing its bactericidal action with that of unheated plasma and whole blood. Heated plasma had practically no bactericidal action on the gonococcus. Heated plasma added to washed cells also resulted in the death of no organisms.

It was conceivable from these observations that the difference between the action of heated and unheated plasma was not due to the inactivation of complement but to the fact that unheated plasma was a poor culture medium whereas heated plasma was a good one. However, from the following experiments, we demonstrated that unheated plasma killed gonococci when antibodies were present, and heated plasma failed to kill them owing to the inactivation of complement.

The first experiment showed that complement was necessary for bactericidal action to take place. We obtained a polyvalent antigonococcal horse serum (Parke Davis) which agglutinated all strains of gonococci used. This immune serum did not contain complement. As a result, it is seen in Table III that the serum alone had no bactericidal power. However, when 0.1 cc. of fresh, undiluted human serum was added to 0.4 cc. of this immune serum, excellent bactericidal action resulted. It is seen further that the same amount of fresh serum (0.1 cc.) diluted to 0.5 cc. did not kill the organisms. Only when the immune serum was activated by fresh serum containing complement did bactericidal action result.

To study further the bactericidal action in human plasma, samples were obtained that possessed killing power, and they were compared with those

TABLE III

*The bactericidal action of immune serum with and without the addition of fresh human serum (complement) \**

	Strain	Dilution of organism							Number of organisms
		10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	
1. Immune serum 0.5 cc.	"C" 0.1 cc.	+	+	+	+	+	+	+	10 <sup>-7</sup> 5
2. Fresh human serum 0.1 cc. Normal salt solution 0.4 cc.	"C" 0.1 cc.	+	+	+	+	+	0	0	10 <sup>-6</sup> 6
3. Immune serum 0.4 cc. Fresh human serum 0.1 cc.	"C" 0.1 cc.	0	0	0	0	0	0	0	10 <sup>-6</sup> 0

\* + = growth.  
0 = no growth.

showing no killing power. The results are recorded in Table IV. Unheated plasma "W" was capable of destroying the "C" strain of gonococcus, whereas the heated serum had lost this capacity. To test whether heating the serum destroyed the antibody, immune serum was added to the heated serum; no killing of the organism resulted. The heated serum was then reactivated by the addition of fresh serum from a normal individual, and the killing power was restored in part. From this experiment, it would appear that heating a plasma that was capable of killing gonococci inactivated complement and did not destroy antibody. The addition of immune serum to heated serum was ineffective in restoring bactericidal power, whereas the addition of fresh serum (complement) restored it in part.

In the case of serum "K" which contained no antibodies against organisms "C" it was found that the addition of antibody (immune serum) caused killing when it was added to unheated serum, but no killing was observed when it was added to heated serum. The death of the organisms was produced when immune serum was

TABLE IV

*The bactericidal action of heated and unheated human plasma with and without antibodies \**

Observations	Dilution of organisms								Number of organisms
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-7</sup>	
Serum "W," unheated 0.5 cc. Organism "C" 0.1 cc.	+	0	0	0	0	0	0	5	
Serum "W," heated 0.5 cc. Organism "C" 0.1 cc.	+	+	+	+	+	+	0	5	
Serum "W," heated 0.4 cc. Organism "C" 0.1 cc. and immune serum 0.1 cc.	+	+	+	+	+	+	0	5	
Serum "W," heated 0.4 cc. Organism "C" 0.1 cc. and fresh serum "S" 0.1 cc.	+	+	+	0	0	0	0	5	
Fresh serum "S" 0.5 cc. and organism "C" 0.1 cc.	+	+	+	+	+	+	+	5	
Immune serum 0.5 cc. and organism "C" 0.1 cc.	+	+	+	+	+	+	+	5	
Serum "D," unheated 0.5 cc. Organism "C" 0.1 cc.	0	0	0	0	0	0	0	2	
Serum "D," heated 0.5 cc. Organism "C" 0.1 cc.	+	+	+	+	+	+	+	2	
Fresh serum "S" 0.5 cc. and organism "C" 0.1 cc.	+	+	+	+	+	+	+	2	
Serum "D," heated 0.5 cc. Fresh serum "S" 0.5 cc. and organism "C" 0.1 cc.	+	0	0	0	0	0	0	2	
Serum "K," unheated 0.5 cc. Organism "C" 0.1 cc.	+	+	+	+	+	+	+	4	
Serum "K," heated 0.5 cc. Organism "C" 0.1 cc.	+	+	+	+	+	+	+	4	
Serum "K," heated 0.5 cc. Organism "C" 0.1 cc. and immune serum 0.1 cc.	+	+	+	+	+	+	+	4	
Serum "K," heated 0.25 cc. Immune serum 0.1 cc., organism "C" 0.1 cc. and fresh serum "S" 0.25 cc.	+	+	0	0	0	0	0	4	
Serum "K," unheated 0.5 cc. Immune serum 0.1 cc. Organism "C" 0.1 cc.	+	0	0	0	0	0	0	4	

\* + = growth.  
0 = no growth.

added to heated serum which had been reactivated by fresh serum. The same results were obtained with another serum "D" which was obtained from a patient with gonococcal arthritis.

It would appear to be established that the gonococcus is killed *in vitro* by lysis. Both antibody and complement are necessary for this action.

#### *Bactericidal action of whole defibrinated blood and washed cells suspended in saline*

It has been recognized for many years that the gonococcus is actively phagocytized by polymorpho-

nuclear leukocytes. Whether or not this indicates a method of destruction has remained in dispute. By a series of experiments we set out to determine the relative importance of the cells in the destruction of the gonococcus *in vitro*. In Table V, we present 5 observations comparing the bactericidal power of whole, defibrinated blood with that of washed cells. The cells were washed 4 times in sterile, physiological saline, as described under "Methods." It is plain from this experiment that the suspension of cells alone was not capable of killing the gonococcus. When the cells were removed from the tubes, and smears were made and stained, it was noted that active phagocytosis had taken place. There was, however, no indication that bacterial growth had been suppressed.

TABLE V

*Comparison of bactericidal power of whole defibrinated blood with that of washed cells suspended in saline \**

Observation number	Whole defibrinated blood							Washed cells in saline						
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>
1	0	0	0	0	0	0	0	+	+	+	+	+	+	0
2	+	0	0	0	0	0	0	+	+	+	+	+	+	+
3	0	0	0	0	0	0	0	+	+	+	+	+	+	0
4†	0	0	0	0	0	0	0	+	+	+	+	0	0	0
5	+	+	+	+	0	0	0	+	+	+	+	+	+	+

\* + = growth.

0 = no growth.

† No organisms were present in 6 and 7 dilutions.

#### *A comparison of phagocytosis in specimens of blood with and without bactericidal power*

To supplement the above observations on phagocytosis and the rôle of the polymorphonuclear cells in killing the gonococcus, the degree of phagocytosis was studied in several cases. As has been related in the bactericidal studies of the whole blood, there was evidence that the gonococcus was killed by lysis and not by intracellular digestion. When the killing was complete, it was not possible to find any organisms within or outside the cells. Inasmuch as it was not possible to demonstrate organisms when they were killed, the possibility of intracellular digestion was not absolutely excluded. The experiment with cells alone demonstrated that the organisms were engulfed by the leukocytes but growth was not suppressed, so that it seemed unlikely that the cells actually killed them independently. It was of interest, however, to determine whether there was

TABLE VI

*Comparison of bactericidal power and phagocytic activity of whole blood and washed cells from normal people and patients with gonococcal arthritis \**

Observation	Killing power whole defibrinated blood							Phagocytosis whole defibrinated blood. 50 polys counted		Killing power cells washed in saline							Phagocytosis cells washed in saline. 50 polys counted	
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	With organisms	Without organisms	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	With organisms	Without organisms
Patient 1.....	+	+	+	0	0	0	0	40	10	+	+	+	+	+	+	0	49	1
Control 1.....	0	0	0	0	0	0	0	50	0	+	+	+	+	+	+	0	45	5
Patient 2.....	+	+	+	+	+	+	+	33	17									
Control 2.....	+	+	0	0	0	0	0	30	20									

\* + = growth.  
0 = no growth.

any difference in the opsonic index of patients with good bactericidal power and those with a low titer.

Table VI illustrates the killing power and the phagocytic activity of the whole defibrinated blood of patients with gonococcal infections as compared with the results obtained with the blood of normal controls. It is to be noted that there is no correlation between the bactericidal power and phagocytic activity of the whole blood. The same table also shows the absence of killing power of cells washed in saline; a majority of the cells, however, engulfed organisms. Table VII gives a comparison of the killing power of whole defibrinated blood of normal controls with the degree of phagocytosis present.

It may be taken as established, then, that the opsonic index is no indication of the bactericidal power of a patient's blood. Phagocytosis occurs without killing, and the death of the bacteria can be accomplished without cells.

*The bactericidal effect of polyvalent antgonococcal horse serum when added to whole defibrinated blood of patients with gonococcal infection*

To determine whether the bacteriolytic power of the whole blood could be enhanced by the addition of immune serum, the following experiment was done. A polyvalent antgonococcal horse serum that agglutinated all strains of the gonococcus that we studied was added to the whole defibrinated blood of a patient with gonococcal arthritis. Table VIII illustrates the effect of adding 0.1 cc. of a 1:10 and 1:40 dilution of this serum to 0.5 cc. of defibrinated blood of Patient C. The "C"

TABLE VII

*Comparison of the killing power with the phagocytic activity of whole defibrinated blood of normal controls \**

Observation number	Killing power whole blood							Phagocytosis 50 polys counted	
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	With organisms	Without organisms
1	+	+	+	0	0	0	0	40	10
2	+	+	+	+	+	+	+	46	4
3	+	+	+	+	+	+	0	37	13
4	+	+	+	+	+	+	0	35	15
5	+	+	+	+	0	0	0	44	6
6	+	+	+	+	0	0	0	43	7

\* + = growth.  
0 = no growth.

strain of gonococcus was obtained from this patient, and it is seen that 0.5 cc. of his blood was capable of killing only 7 organisms. Adding the above dilutions of immune serum to his blood greatly increased the killing power. This procedure was repeated successfully several times using

TABLE VIII

*Bactericidal power of whole defibrinated blood and that of the same blood to which small amounts of gonococcal immune serum had been added \**

Number of colonies "C" strain	Whole defibrinated blood of Patient "C"	Whole defibrinated blood + 0.1 cc. 1:10 immune serum	Whole defibrinated blood + 0.1 cc. 1:40 immune serum
700,000.....	+	+	+
70,000.....	+	+	0
7,000.....	+	0	0
700.....	+	0	0
70.....	+	0	0
7.....	0	0	0

\* + = growth.  
0 = no growth.

the blood of both normals and patients with gonococcal infections. The antigonococcal horse serum did not contain complement and, therefore, had little or no killing power. Its bactericidal action depended upon the addition of fresh serum containing complement.

*The bactericidal effect of whole defibrinated blood of patients before and after the intravenous administration of polyvalent antigonococcal horse serum*

As related above, it was found that when polyvalent, antigonococcal horse serum was added to whole defibrinated blood *in vitro*, the bactericidal power of the blood was greatly increased. We then proceeded to administer the serum intra-

chart, were similar to those shown in the figure. When 0.1 cc. of a 1:10 and 1:40 dilution of serum was added to the patient's blood *in vitro*, the killing power was increased. By calculation it was found that if 0.1 cc. of a 1:40 dilution of immune serum added to 0.5 cc. of his blood killed all the organisms except those in the first dilution, approximately 25 cc. of undiluted serum would have to be administered intravenously to produce the same effect *in vivo*. Accordingly, 25 cc. of polyvalent, antigonococcal horse serum were administered intravenously without reaction. Four hours later, a bactericidal test showed an increase of the killing power of the patient's blood. Twenty-four hours later, the killing power had reached the same level as that found when the

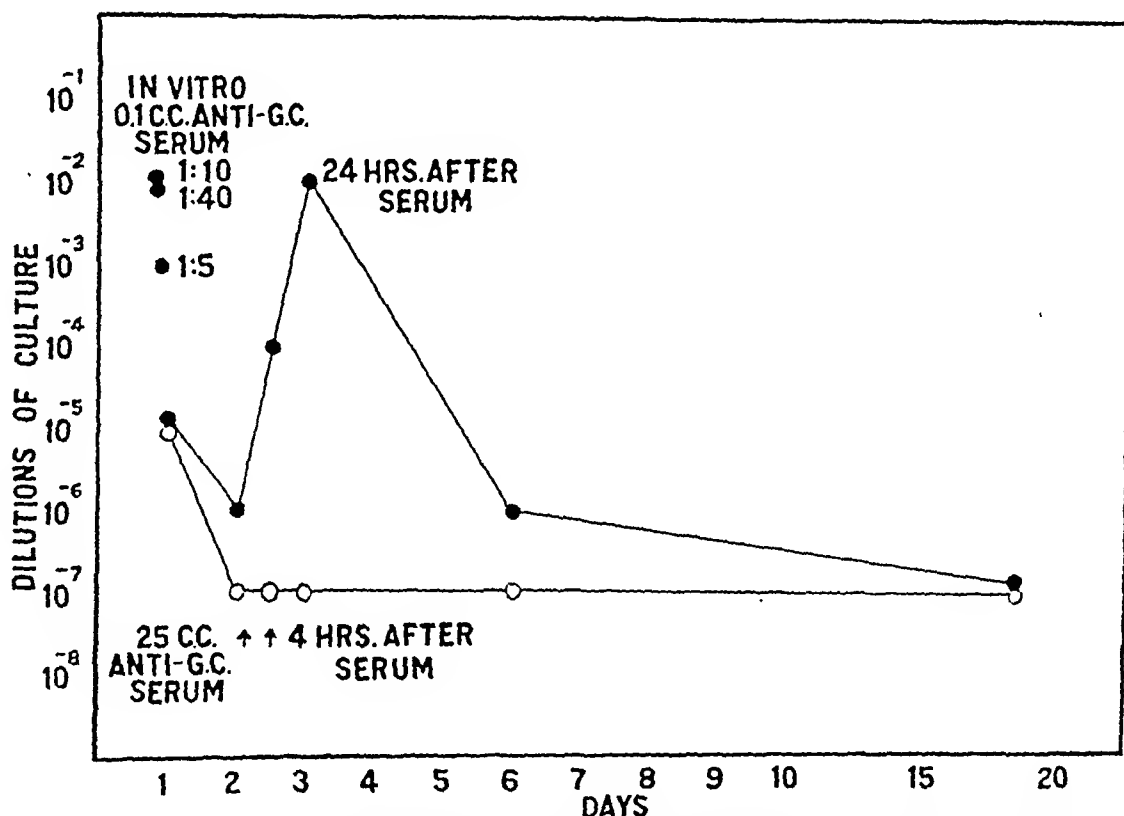


FIG. 1. THE BACTERIOLYTIC POWER OF THE WHOLE BLOOD FOLLOWING THE ADDITION OF IMMUNE SERUM *in vitro* AND *in vivo*.

venously to a patient for the purpose of determining whether the same effect could be obtained *in vivo*. The patient was recovering from an attack of acute gonococcal arthritis. The strain of gonococcus was obtained from his prostatic discharge. In Figure 1, it is seen that the killing power of the patient's blood was essentially the same as that of a normal control. The results of several other control tests, not included on the

equivalent amount of serum was added *in vitro*. This maximum bactericidal action was of short duration, however, and 5 days after receiving the serum, the killing power of his blood had returned to the level observed before injection.

An opportunity arose for a practical demonstration of the value of immune gonococcal serum in a patient with gonococcal septicemia. The focus of his infection was a chronic prostatitis; he did

not have an endocarditis. His illness was further complicated by cirrhosis of the liver with jaundice and ascites, and a hemorrhagic eruption of the whole body due to a thrombocytopenia. Before the administration of immune serum, gonococci were cultured from his circulating blood, and the killing power of his blood was less than that of the normal control. Immune serum was then administered intravenously, and the following day his blood was sterile, and there was an increase in the titer of antibodies in the blood. On the basis of the preceding observation that this increase in bactericidal power resulting from serum treatment may be of only short duration, daily injections of serum were administered. The patient's blood continued to show excellent killing power. From these results, it seems obvious that the bactericidal power of the blood can be enhanced *in vivo* and *in vitro* by the addition of antigonococcal immune serum. The therapeutic significance of these observations will be discussed in a subsequent paper.

#### DISCUSSION

From the evidence that we have presented, there can be little doubt that whole blood from most patients with gonococcal infection is bactericidal for the gonococcus. This property is a function of the plasma or serum and it produces its action by means of lysis. Bacteriolysin and complement are necessary for this mechanism of destruction. There was no evidence that the polymorphonuclear leukocytes were able to kill gonococci *in vitro*. This was the case, in spite of the fact that active phagocytosis could be demonstrated. In this respect, it would appear that the mechanism for the destruction of the gonococcus is similar to that of other gram-negative organisms. Ward and Wright (2) in a similar study of the *B. influenzae* state, "Generally speaking, the gram-positive organisms are first sensitized by the antibody in the serum and then phagocytized by the leukocytes, no organisms being killed in the absence of the cells; but with the gram-negative organisms, the cells play a very minor rôle, the bacteria being sensitized by the antibody and then killed by the complement. Under certain conditions the sensitized organisms are not only killed, but undergo lysis. *B. influenzae* falls

into this category." Topley (3) is of the same opinion in regard to such organisms as the cholera vibrio, the typhoid bacillus, and "most gram-negative organisms." In an immunological study of the meningococcus, which is closely related to the gonococcus, Silverthorne and Fraser (4, 5) concluded that the plasma of human blood, and not the cells, killed the meningococcus. The earlier work of Torrey (6) is of considerable significance in relation to immunological studies done on guinea pigs inoculated with gonococci. He concluded that phagocytosis played little part in the destruction of gonococci, since the degree of phagocytosis was apparently the same whether the animal died or recovered. Torrey believed that the rapid destruction of gonococci in the blood was due to specific bactericidal bodies. Further important observations were made by Martin (7) in studying the bactericidal action of an animal's blood upon the gonococcus; he stated that complemented immune serum was most important in the destruction of gonococci.

Although the circulating leukocytes, particularly the polymorphonuclear leukocytes, do not appear to play an important part in killing the gonococcus, it would be desirable to know more concerning the tissue cells and their action on the gonococcus. Torrey (6) studied the phagocytic activity of cells in peritoneal exudates and of the omentum in guinea pigs injected intraperitoneally with gonococci. He stated that macrophages in the peritoneal exudate ingested gonococci with much greater avidity than did the polymorphonuclear leukocytes on the surface of the omentum. But the degree of phagocytosis was the same whether the animal died or recovered.

Our information concerning the significance of tissue reactions in the prevention of spread in gonococcal infections is very meager. An early reaction to infections of the urethra is a profuse exudate consisting mostly of polymorphonuclear leukocytes. What action do these cells have on the organism, especially those containing intracellular gonococci? If the organisms are not killed when ingested by the leukocytes, it might be postulated that this phagocytosis is an attempt to localize the gonococci and prevent their dissemination throughout the tissues. Further immunological studies are necessary before a proper eval-

uation can be made concerning the rôle of the tissue cells and those found in exudates in protecting the body against widespread invasion by gonococci.

#### SUMMARY

1. A study of the bactericidal action of whole defibrinated blood on several strains of gonococci justifies the following conclusions.

*a.* Human defibrinated whole blood has the property of killing gonococci by means of lysis.

*b.* This bactericidal factor resides in serum or plasma, and is dependent upon the presence of complement.

*c.* Heated serum or plasma, or immune serum without complement, have little or no bactericidal power.

*d.* Freshly washed leukocytes suspended in saline have little or no killing action, though the polymorphonuclear leukocytes actively phagocyte the organisms.

*e.* There does not appear to be any relationship between the degree of phagocytosis and the bactericidal power of blood.

2. The addition of polyvalent, antigonococcal horse serum to human blood greatly increases the killing power.

3. The intravenous administration of immune serum to patients greatly increases the killing power of their blood.

We acknowledge our thanks to Miss Marjorie L. Jewell and Miss Eleanor M. Fleming for technical assistance.

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# STUDIES OF GONOCOCCAL INFECTION. II. THE BACTERIOLYTIC POWER OF THE WHOLE DEFIBRINATED BLOOD OF PATIENTS WITH GONOCOCCAL ARTHRITIS<sup>1</sup>

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In the previous paper (1), we presented evidence that the bactericidal action of whole defibrinated blood for the gonococcus was the same as that of plasma or serum. It was demonstrated that phagocytosis of the gonococcus by the polymorphonuclear leukocytes played little or no part in the destruction of the organisms *in vitro*. The experiments supported the conclusions that the gonococcus was killed *in vitro* by bacteriolysis, and that this was accomplished by a sensitization of the organisms by antibody; the lysis was completed by the action of complement. There was no evidence that intracellular digestion of the organisms by leukocytes took place, in spite of the fact that active phagocytosis could be demonstrated.

We then proceeded to study the variations in the bactericidal action of the whole defibrinated blood of 29 patients with gonococcal infections. Sixteen had local infections, notably urethritis, endocervicitis, or conjunctivitis, and the remaining 13 individuals had gonococcal arthritis.

## METHODS

The bactericidal action of whole blood was determined according to the method described in the preceding paper (1). A control was included at the time of each test so that the patient's blood could be compared with the blood of a non-infected individual. The same control was always used for a given patient. In all, there were 210 tests carried out on patients and 220 on controls. For purposes of discussion we have divided the cases into those with local lesions and those with arthritis. In order that the bacteriolytic power of a patient's blood could be determined against his own strain bacteria as well as other strains, the tests in some patients were car-

ried out with more than one strain of gonococci. The same procedure was carried out with different controls.

The results are recorded in accordance with the number of organisms killed by 0.5 cc. of blood. That is to say, if there was growth in the tube containing 0.1 cc. of a  $10^{-1}$  dilution of the culture and none in the tube containing 0.1 cc. of the  $10^{-2}$  dilution, it was first recorded as killing power in  $10^{-2}$ ; then the number of organisms was calculated from the quantitative cultures in the dilution of  $10^{-6}$ . For example, if 1 cc. of a  $10^{-6}$  culture contained 10 organisms, then the number contained in the 0.1 cc. of the  $10^{-2}$  dilution would be 10,000 organisms.

## RESULTS

### *Bacteriolytic titer of the whole defibrinated blood of patients with local lesions and arthritis*

1. Controls. *Patient's organism versus whole defibrinated blood of controls.* One-half a cubic centimeter of a normal individual's whole blood was mixed with different dilutions of a 24-hour ascitic fluid broth culture of the gonococcus isolated from the patient and incubated as described under methods of study. The same individual was used as a control every time the patient's blood was studied. In this way a number of observations were obtained that permitted one to determine the fluctuation of the titer of the normal blood from time to time, and to compare the bacteriolytic action of non-infected individuals with that of patients with infections. We also studied the bacteriolytic action of the same blood against several different strains. In addition, in some of the cases, the bacteriolytic action of several controls was tested for the same organism. This was done in order to determine the difference in the bacteriolytic titer of the blood of various normal individuals for the same strain. It was found that the titer of the normal individual's

<sup>1</sup> This investigation was aided, in part, by a grant from the Milton Fund and Clark Bequest.



blood did not fluctuate more than a 100-fold dilution, and in many instances it was either constant or varied less than 100-fold. There was evidence, however, that the titer varied for individual strains. The results are summarized in Tables I and II. From Table I, it can be seen that the blood of normal individuals, when tested against the majority of strains of gonococci, could only kill relatively small numbers of organisms, although there were a few susceptible strains of which more than 10,000 were killed by 0.5 cc. of blood. That this was due to the variations in the strains of organisms rather than variations in the activity of blood from different individuals is borne out by the information summarized in Table II. Here, it is ascertained that the variations in the bacteriolytic power of the blood of the different controls for single strains was slight. There were a few exceptions, but from these observations it would seem justifiable to say that control bloods were able to kill varying numbers of gonococci, depending upon the strain and the individual. In general, the control bloods, 0.5 cc., were able to kill less than 100 organisms of 65 per cent of the strains tested. It was also found that the controls were able to kill more organisms derived from patients with local lesions than those obtained from patients with arthritis. Insofar as one can determine from experiments of this kind, it may be contended that certain normal individuals possess natural bacteriolysins against some strains of gonococci. It cannot be maintained

TABLE I

*Maximum number of gonococci killed by 0.5 cc. of blood from controls*

Control number	Number of strains studied	Less than 100	100 to 10,000	Over 10,000
1	25	17	6	2
2	11	7	2	2
3	8	8	0	0
4	5	4	1	0
5	4	4	0	0
6	4	3	1	0

from such studies alone, however, that the organisms vary in their ability to invade the tissues freely, although it is suggestive that strains which cause arthritis are less often killed in large numbers by normal individuals.

## 2. Patient's organism and whole defibrinated

TABLE II

*Maximum number of gonococci killed by 0.5 cc. of blood from various controls when the same strain was used*

Strains	Controls					
	1	2	3	4	5	6
1	10	25		25	1	7
2	10	3	100	8	3	3
3	18	7	300	7	18	2
4	1,400	35	35	7	18	2
5	400,000	97,000			9,700	9,700
6	1	25				
7	700	150				
8	6		5			
9	6		3			
10	400		800			
11	1,000		10			
12	100,000		40,000			

*blood of patients.* When the bacteriolytic titer of the patients' blood was studied in relation to the organism that had been isolated from them, it was found that the number of organisms that were killed by the blood varied considerably. The results can be discussed more easily by dividing the cases into two groups: *A*, those with local lesions; *B*, those with arthritis.

*A. Patients with local lesions.* The results of the studies of the bacteriolytic titer of the blood are shown in Figure 1. The maximum number of organisms that were killed by the patient and the controls during the period of observation are charted. Specimens of blood from five of the sixteen patients were capable of killing many more organisms than were those from controls, whereas in the remaining eleven instances the titers of specimens from the patients and controls were approximately the same. These observations indicate that a local infection may be caused by an organism that can be killed in large numbers by normal, non-infected individuals. Moreover, when an infection takes place with an organism which cannot be killed in large numbers by normal individuals, it is common to observe an increased bacteriolytic titer of the patient's serum as the disease advances. In Figure 2 the bacteriolytic titer of the blood of 3 patients with local lesions is charted.

*B. Patients with arthritis.* When the blood of patients with arthritis was studied and the results charted (Fig. 3), it was found that 8 of the 13 patients had a higher bacteriolytic titer than did

# GONOCOCCAL INFECTION. II

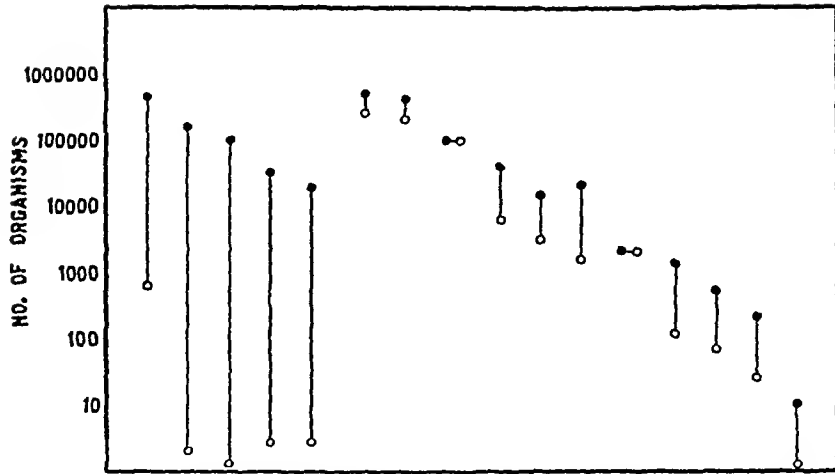


FIG. 1. THE MAXIMUM NUMBER OF ORGANISMS KILLED BY 0.5 CC. OF WHOLE DEBRINATED BLOOD DURING THE NATURAL COURSE OF LOCAL GONOCOCCAL INFECTIONS

The dots represent the maximum number killed by the patients, the circles the maximum number killed by normal controls. Each specimen of blood was tested against the organism derived from the patient.

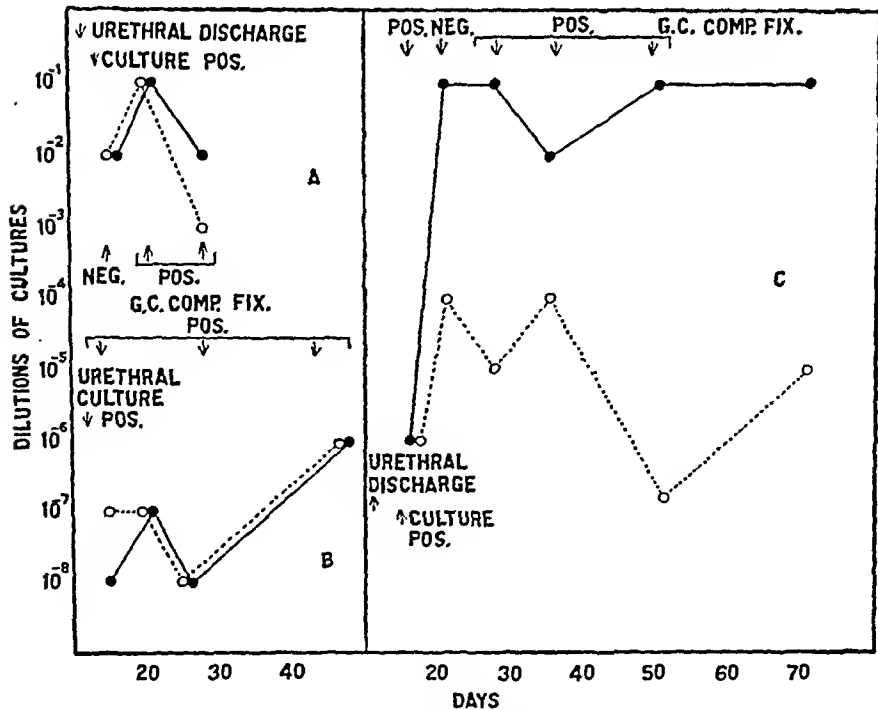


FIG. 2. THE BACTERIOLYTIC TITER OF THE BLOOD IN 3 PATIENTS WITH GONOCOCCAL URETHRITIS

○-----○ Control.  
●-----● Patients.

the controls, the remaining 5 were unable to kill many more organisms than the controls. It is worthy of comment, however, that 0.5 cc. of blood from our controls was incapable of killing more than 10,000 organisms. When organisms from urethritis were studied, it was found that a number of controls were able to kill more than 10,000 of these organisms. This is emphasized more clearly in Table III. This table was constructed from the results of 210 bactericidal tests in 29 patients and from 220 tests in a group of controls. The bacteriolytic power shown in the table represents the maximum number of organisms that were killed during the course of the disease. The bacteriolytic power of the patient's blood was determined against his own strain. The same control was always used for a given patient and the

TABLE III

*Maximum number of gonococci killed by 0.5 cc. of blood from patients and controls*

	Total number	Less than 100	100 to 10,000	Over 10,000
Patients with arthritis.....	13	0	8	5
Control observations.....	13	10	3	0
Patients with local lesions.....	16	2	4	10
Control observations.....	16	9	5	2

killed by normal control plasmas. It is evident, then, that an active arthritis may be present with or without a high bacteriolytic titer, but it would seem that the organisms in patients from arthritis are more likely to be resistant to destruction by the blood of normal individuals.

We have already stated that the bacteriolytic

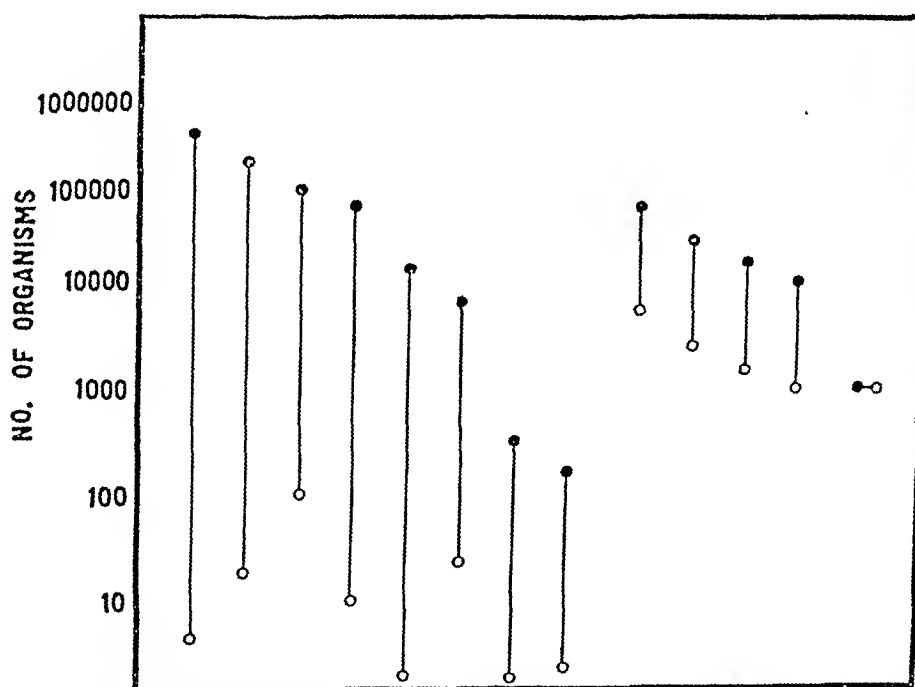


FIG. 3. THE BACTERIOLYTIC TITER OF THE WHOLE BLOOD IN PATIENTS WITH GONOCOCCAL ARTHRITIS. THE MAXIMUM NUMBER OF ORGANISMS KILLED BY 0.5 CC. OF WHOLE DEFIBRINATED BLOOD DURING THE NATURAL COURSE OF GONOCOCCAL ARTHRITIS

The dots indicate the maximum number killed by the patients, the circles, the maximum number killed by normal controls. Each specimen of blood was tested against the organism derived from the patient.

organism was derived from the patient. From the results shown in Figure 3 and Table III, it is fair to say that patients with gonococcal arthritis usually have a higher bacteriolytic titer in the blood plasma than do normal controls. Moreover, very few organisms of these strains are

titer of the blood plasma increased during the course of the illness in some patients with either local infections or arthritis, and while this titer may be maintained for several weeks, it frequently falls off gradually (Fig. 4). It was of interest, therefore, to compare the titer of the

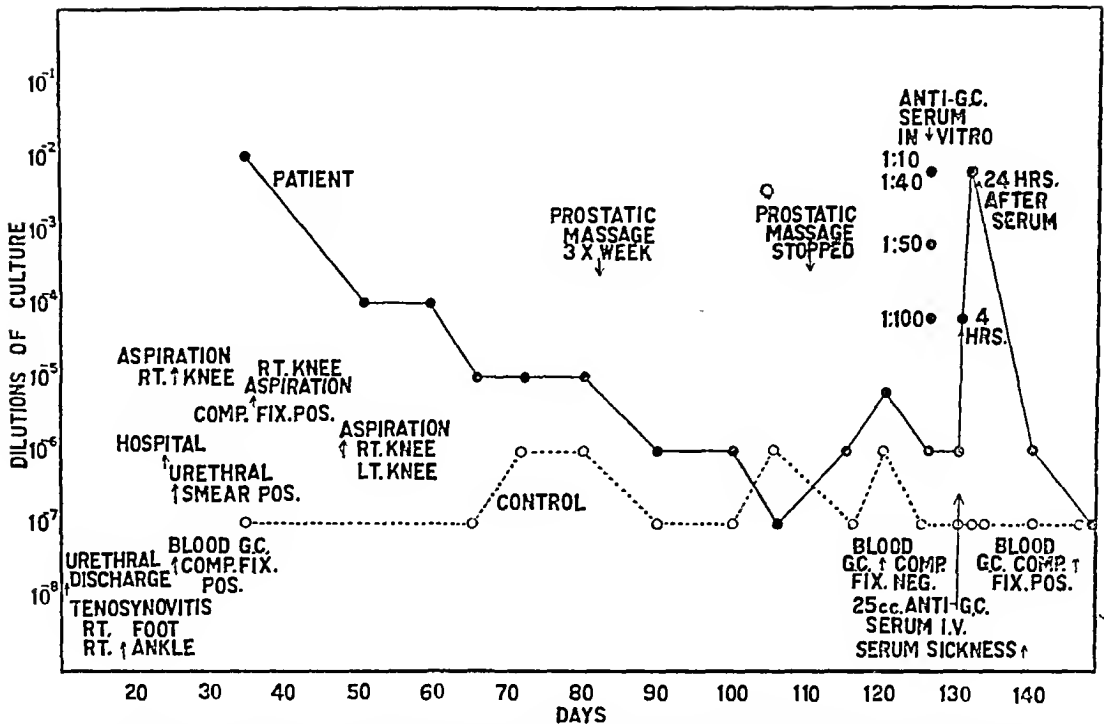


FIG. 4. THE COURSE OF THE BACTERIOLYTIC TITER OF THE WHOLE DEFIBRINATED BLOOD IN A PATIENT WITH GONOCOCCAL ARTHRITIS, SHOWING THE EFFECTS OF INJECTING SERUM INTRAVENOUSLY

blood from several patients against their own organism and against strains derived from other patients. The pertinent information is tabulated in Table IV. The patients' blood was capable of killing many more of their own organisms than other strains. There were, however, several exceptions. Strain number 5 was obtained from a case of urethritis, and specimens of blood from all of the controls and patients were able to kill large numbers of these organisms. It appears,

then, that the immune reactions tend to be specific, although there are some virulent strains which are killed in large numbers by blood from normal as well as from individuals infected with gonococci.

#### Complement fixation

Repeated complement fixation reactions were done on the blood serum of the patients. Our previous experience (2) indicated that the gonococcal complement fixation test was of value in the diagnosis of gonococcal arthritis, inasmuch as it was positive in 85 per cent of the cases studied. In this study, it was found to be positive in 11 of the 16 patients with local infections and in 10 of 15 patients with arthritis.

The reactions were positive in some patients as early as 7 days after the onset of the signs of acute infection, and in a few it appeared before there were any signs of an increased bacteriolytic titer in the blood. In several it was possible to demonstrate a positive complement fixation reaction when the bacteriolytic titer failed to increase. The two reactions did not parallel each other, but it was not uncommon to find a positive

TABLE IV

Comparison of patient's bacteriolytic titer against his own and other strains of gonococci\*

Patient number	Own strain	Other strains				
		1	2	3	4	5
1	500	720	140			20,000
2	13,000	6,000		3	96	200,000
3	720,000		1	3		200,000
4	510,000	7	130	6		
5	10	35	3	0		97,000
6	70,000	3	7	2	5	3,100
Controls		2	3	3	6	400,000

\* The number of organisms indicates the maximum number killed in 0.5 cc. of whole blood.

complement fixation test in a patient who was infected by a strain that could be killed in large numbers by the blood of controls. It is indicated from our observations that this test was helpful in diagnosis, but it was of little value in assessing the immune reactions in a quantitative fashion.

Once the complement fixation test becomes positive it may remain so for as long as 3 months. Indeed, we have observed some cases in which it was positive one year after an acute arthritis had subsided.

*Agglutination reactions*

The blood serum from the patients was tested for agglutinins against homologous organisms at various intervals of time during the course of the disease. In no case did we observe the appearance of agglutinins. Thus, the use of the agglutination test as a means of diagnosis or as a method for the study of immune reactions in patients was of no assistance.

*Titration of complement in patients' blood*

In the previous paper (1) referred to, it was demonstrated that complement was necessary for the lysis of organisms after they had become sensitized by the antibody. The titer of complement was determined in the blood serum of patients with gonococcal arthritis. The purpose of this was to detect fluctuations from the normal. In other studies of complement we found that normal individuals did not show fluctuations of more than 0.1 cc. of complement on repeated examinations although the differences in titer from one individual to another were greater than 0.1 cc. The complement titer varied from 0.02 to 0.09 cc. in all examinations but one. In this instance it was 0.2 cc. Moreover, during the course of the disease, the widest fluctuation in any single case was from 0.2 cc. to 0.07 cc. It is clear, then, that there is an adequate amount of complement present in the blood of people with gonococcal infections to complete the lysis of organisms, provided antibody is available.

DISCUSSION

From the data presented, there is a fair amount of evidence that the blood of normal individuals is capable of destroying varying numbers of some

strains of gonococci isolated from different types of gonococcal infections. It is more common for the blood of normal individuals to destroy large numbers of organisms obtained from local lesions than it is to kill large numbers of bacteria isolated from patients with arthritis. Observations of this kind would indicate that some strains are more invasive than others and while invasiveness cannot be correlated entirely with the absence of antibodies in the circulating blood, it does not appear unlikely that the presence or absence of bacteriolytic antibody in the blood is a factor of importance. It should be added, however, that infection with an organism that can be killed in moderate numbers by the blood of normal individuals may be followed by arthritis, but, in our experience at least, it was somewhat more common to observe arthritis when the organisms were not killed in large numbers by the blood of normal individuals. Attention should be called to the fact that active infection of the urethra can continue in the presence of an excellent bactericidal titer of the blood; this may also be said of the cases in which the joints are involved.

When patients with arthritis and those with local lesions without arthritis are studied for differences in bacteriolytic titer, it becomes manifest that those with arthritis develop a higher titer for their organism more often than do those with only a local lesion. This is what might be expected, since in other infections it is the rule to observe higher bactericidal power in the blood when metastatic lesions are present.

The complement in the blood of patients with gonococcal arthritis was at the same level as it is in normal individuals, so that there is no evidence of a lack of this substance which is so important in the antigen-antibody reaction. The complement fixation test was found to be of aid in diagnosis but it could not be correlated with the titer of the bacteriolytic test. Information was obtained indicating that the complement fixing antibodies appeared earlier than did other antibodies.

The agglutination reaction was negative in all cases and was, therefore, of no aid in diagnosis.

It is not possible, from these studies alone, to state the relative importance of bacteriolysins and local tissue reactions in the mechanism of recovery from gonococcal arthritis. The factors that localize the organisms, suppress their growth, and

finally destroy them are probably multiple. There can be no question, however, that active infection may go on in the face of a high bacteriolytic titer of the plasma, and it is also obvious that invasion from the local focus does not always take place when one fails to demonstrate bacteriolytic substances in the blood. When bacteriolysins are present in the blood serum they undoubtedly aid in the destruction of organisms and probably assist in the localization of infection. The process of lysis is certainly operative to some extent when the organisms localize in an area that can be reached by the blood plasma in large amounts, such as occurs in the case of arthritis. The precise mechanism for the destruction of organisms in various tissues remains obscure. The recent important studies of McMaster and Hudack (2) on the local formation of antibodies in lymph nodes are of significance in emphasizing the importance of antibody formation in tissues before they can be demonstrated in the circulating blood. Such observations suggest, at least, that the destruction of organisms in some tissues is due in part to the development locally of specific antibodies. There is no doubt that the appearance of antibodies in the circulating blood can be taken as an indication of their formation elsewhere and of their existence in some tissues. There is abundant evidence that a part of immunity to infections depends on specific antibodies and that these antibodies have a profound effect on local cellular reactions. It is not possible to say at present how much of a rôle fixed antibody in the tissues plays in the defense mechanism in gonococcal infections; but we have no reason for assuming that it does not play a part since recovery from local infections may occur without the demonstration of specific bacteriolysins in the circulating blood. For the above reasons, it does not seem improper to regard the presence of bacteriolysins as an immune response of significance in the destruction of organisms, in spite of the fact that their relative importance in the mechanism of recovery cannot be evaluated with precision at the present time.

In order to explain the appearance of arthritis in cases of gonococcal infection, it is necessary to understand how the organisms invade and why they survive in the synovial tissues. The present investigation suggests that invasion of the tissues

is dependent in part upon: 1, the virulence of individual strains; 2, the antibody content of the host's blood. There are undoubtedly other factors which are not well defined, notably the effects of local inflammation and the rôle of the leukocyte in the fixation of the infection. Why the organisms seem to survive in the synovial cavities will be taken up in a subsequent paper.

#### SUMMARY AND CONCLUSIONS

1. The whole blood from normal individuals is capable of destroying varying numbers of gonococci obtained from patients with infection. Strains of gonococci obtained from local lesions, such as urethritis, are often killed in larger numbers than are those derived from patients with arthritis.

2. Destruction *in vitro* occurs by lysis and this is a function of the blood plasma and not of the polymorphonuclear leukocytes.

3. During and following the course of a gonococcal infection, especially arthritis, there is evidence of an increase in the bacteriolytic titer of the blood plasma. This is an immune response that can be regarded as an aid in the destruction of the organism.

4. Patients tend to develop a higher bacteriolytic titer in their serum against their own organism than against other strains.

5. The gonococcal complement fixation reaction is a valuable method in the diagnosis of arthritis caused by the gonococcus.

6. The titer of complement of the blood serum is not depressed during the course of gonococcal arthritis.

7. Agglutination tests were of no value in diagnosis since in our experience they were invariably negative.

8. The possible significance of bacteriolysins in gonococcal infections is discussed.

We acknowledge our thanks to Miss Marjorie L. Jewell and Miss Eleanor M. Fleming for technical assistance.

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# THE CLINICAL USES OF HUMAN SERUMS PRESERVED BY THE LYOPHILE PROCESS

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The development of improved technical procedures for preservation, pooling and concentration have considerably facilitated the utilization of human serums in the prevention and treatment of infectious diseases. Full accounts of these procedures have already been published (1, 2), and preliminary reports of their clinical applications have been presented before the Society for Pediatric Research (3) and the American Pediatric Society (4). In brief, the serums used are pooled and preserved by drying *in vacuo* from the frozen state; the dry porous material resulting is called "lyophile" serum. It may be redissolved in as little as one-quarter its original volume of liquid.

The studies here presented consider the problem of human serum therapy with special reference to the use of serum preserved by this process in the prophylaxis and treatment of certain of the more common infectious diseases. The following list summarizes the diseases studied and the serums used:

1. Scarlet fever .....a. Convalescent serum in prophylaxis and treatment.  
b. Pooled adult serum in prophylaxis.
2. Measles .....a. Convalescent serum in prophylaxis.  
b. Pooled adult serum in prophylaxis.
3. Mumps .....a. Convalescent serum in prophylaxis and treatment.  
b. Pooled adult serum in prophylaxis.
4. Chickenpox .....a. Convalescent serum in prophylaxis.  
b. Pooled adult serum in prophylaxis.
5. Erysipelas .....a. Convalescent serum in treatment.
6. Whooping cough .....a. Serum from vaccinated individuals in prophylaxis and treatment.  
b. Pooled adult serum in prophylaxis.
7. German measles .... Pooled adult serum in prophylaxis.
8. Acute hemolytic streptococcal infections .. Convalescent scarlet fever serum in treatment.

It has not been possible to obtain suitable control groups for the clinical evaluation of the serum. Since on hospital wards it is desirable to prevent any secondary cases of the disease in question, it is impracticable to utilize alternate cases as controls. Many of the cases came from private practice and here, likewise, it is impossible to select alternate cases for injection. From general clinical experience certain assumptions must be made concerning the probability of occurrence

of an infectious disease in a particular individual according to the type of disease, the intimacy of exposure, and other well-recognized factors.

It is well recognized that such factors as individual susceptibility and exact duration and degree of exposure, as a rule, cannot be accurately determined with respect to measles, mumps and chickenpox. It is possible to determine with considerable accuracy the susceptibility of an individual to scarlet fever by means of the Dick test. In hospital practice, the presence of a positive Dick test has generally been determined prior to the administration of serum for scarlet fever prophylaxis, but in private practice families in many instances are unwilling to wait the twenty-four hours required for the Dick test before the serum is given.

In passive immunization against measles and at times also against chickenpox, the practitioner desires modification of the disease rather than complete protection. It is not clear in certain cases included in the present studies whether attenuation or complete protection was desired, but in most instances the physician's desire could be determined. Attenuation of the disease was usually preferred.

It has frequently been emphasized that the duration and degree of exposure on a hospital ward, even without special infectious precautions, are far less than in the home where the children usually play together "ad lib." For this reason the cases have been divided into hospital and home groups, since the proportion of expected secondary cases in the home exceeds that to be expected in the hospital.

Data on private cases are secured through the use of protocol outlines which are given to the attending physician when he applies for serum.<sup>1</sup>

<sup>1</sup> We are indebted to Dr. John McK. Mitchell and Dr. Theodore S. Wilder for a large number of the case reports coming from outside of hospitals.



The dosages recommended by various workers in prophylaxis against the contagious diseases for which these serums are given, vary considerably. At first, in the present studies, the dosage for all the infectious diseases was based upon cubic centimeters of serum per year of age. The standard used was 2 cc. per year of age of convalescent serum and 4 cc. per year of age of pooled adult serum. Later, it was found that some of the younger children were not receiving sufficient serum whereas older children were given more serum than was necessary. While the standard of dosage now used is probably far from being exact, according to age or weight, it is simple, requires only two sizes of serum containers (10 and 15 cc.), and thus far has given satisfactory results. The present standard of dosage follows:

Age under 6 years	.....convalescent serum	10 cc.
Age 6 to 12 years	.....convalescent serum	15 cc.
Age over 12 years	.....convalescent serum	20 cc.
Age under 6 years	.....pooled adult serum	20 cc.
Age 6 to 12 years	.....pooled adult serum	20 to 30 cc.
Age over 12 years	.....pooled adult serum	30 to 40 cc.

From the results outlined below these doses are considered protective.

In practically all instances, serum is given in doubly concentrated form; i.e., a dose of 30 cc. of serum is given intramuscularly in a total volume of 15 cc. If desired, this may be even further concentrated to 8 or 10 cc. by reducing correspondingly the volume of distilled water used to dissolve the processed serum.

#### *The prophylaxis and treatment of scarlet fever*

Pathological changes in severe scarlet fever, quite apart from tissue invasion and suppuration, have been noted by a long series of authors. Both the earlier literature and extensive autopsy material have recently been reviewed by Brody and Smith (5). The underlying lesion is one of vascular injury with a concurrent perivascular round cell infiltration. These changes were found in from 75 to 95 per cent of the hearts, livers, kidneys, adrenals and spleens and to a variable degree in the other viscera of the fatal cases studied by Brody and Smith. Lesions resembling those described in scarlet fever have been produced experimentally by Hitchcock, Camero and Swift (6) by the intravenous injection of indifferent streptococci into rabbits previously sensitized to streptococci. Whether the characteristic vascular injury

in scarlatina is due to circulating streptococcal toxins, as suggested by Brody and Smith, or is allergic as in the case of the experimental lesions of Hitchcock, Camero and Swift, the fact of definite visceral pathological changes in this disease is important and deserves emphasis in view of the current tendency to refer to the mildness of scarlatina and its low mortality.

Convalescent scarlet fever serum as a prophylactic measure in exposed individuals has been reported by Degkwitz (7), Dick and Dick (8), Meader (9), Gordon (10), Hoyne, Levinson and Thalheimer (11) and others. A summary of the results of these authors shows that of nineteen hundred and thirty-three contacts given convalescent scarlet fever serum forty-eight or 2.5 per cent developed the disease. Meader reported that in four hundred and fifty contacts treated with convalescent serum 2.9 per cent developed the disease, whereas in a control group of three hundred and twenty-one contacts not given serum, 12.8 per cent developed scarlet fever.

In spite of the fact that convalescent serum is apparently quantitatively low in antitoxin (12), it has been found in the studies here presented that the majority of cases given the Dick test following the injection of convalescent scarlet fever serum show a quantitative reduction in the Dick reaction if not a complete reversal from positive to negative. In seventeen individuals retested every three to four days for two weeks to a month following the injection of convalescent serum, the average trend showed a change from the positive range to the negative range within the first three days, remaining in the negative range for ten to twelve days and then returned to the positive range. This is graphically demonstrated in Figure 1.<sup>2</sup> These findings suggest that a solid protection from an injection of convalescent scarlet fever serum may be expected to last about ten to twelve days, from which time there is a diminishing resistance over two to three weeks.

Included in the present report is a group of forty-seven individuals exposed to scarlet fever

<sup>2</sup> The serums used in the cases represented in Figures 1 and 2 were found by Dr. L. J. Wenger to contain less than 10 National Institute of Health units of antitoxin per cc. of serum in comparison with 900 National Institute of Health units of antitoxin per cc. in antiscarlatinal horse serum.

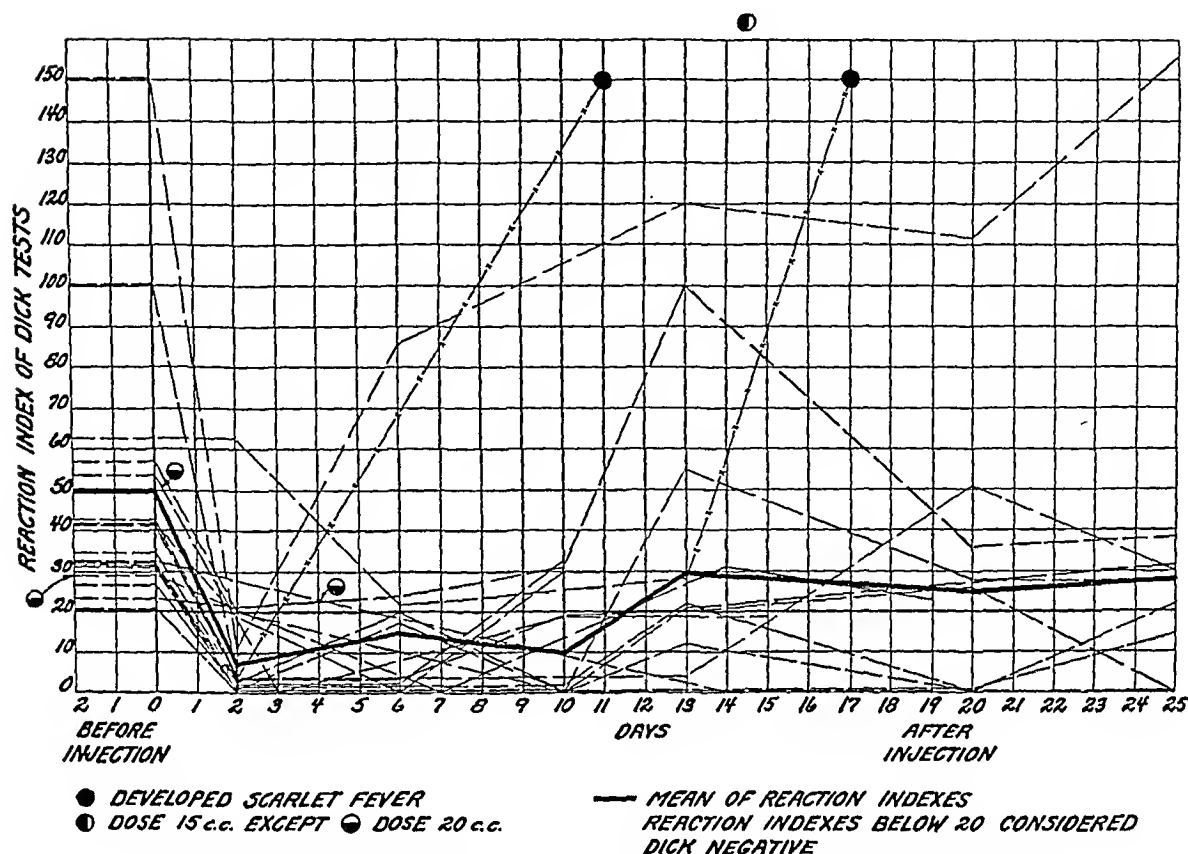


FIG. 1. EFFECT OF CONVALESCENT SCARLET FEVER SERUM ON THE DICK TEST—17 CASES

The reaction index of the Dick tests is determined by adding the two largest diameters of the Dick reaction which are at right angles to each other and by multiplying this sum of the diameters by the following factors which are measurements of intensity: F (faint) = 1; M (medium) = 1.5; B (bright) = 2; when the medium or bright reactions were also swollen or indurated, an additional 0.5 was added to the intensity factor.

who were given pooled adult serum from Dick negative individuals as a prophylactic measure. Of this group two (Fig. 2) developed very mild scarlet fever. Thirty-three of these individuals were known to be Dick positive and were subjected to a very long and intimate exposure to the disease. As will be seen from Figure 2 such pooled adult serum will likewise produce a quantitative reduction in the Dick test.<sup>3</sup>

It would appear on first examining this chart that the pooled adult serum was more effective in altering the Dick test than the convalescent scarlet fever serum. It is our opinion that this is a false impression, probably due to the fact that

those individuals who were given pooled adult serum had, on the average, Dick tests of less intensity prior to serum injection than did those who were given convalescent serum. It is suggested, however, that, in the absence of convalescent scarlet fever serum, pooled adult serum from individuals with negative Dick tests may be of prophylactic value.

Four hundred and fifty-three individuals, for the most part children, received lyophile convalescent scarlet fever serum following exposure to the disease. One hundred and ninety-three of these cases were known to be Dick positive prior to the serum injection. It is unfortunate that a preliminary Dick test was not carried out in more of the individuals reported. In a disease with such a short incubation period as scarlet fever, parents

<sup>3</sup> Presumably the Dick reactions would have been the same immediately preceding the injection of serum as they were when performed 2 days previously.

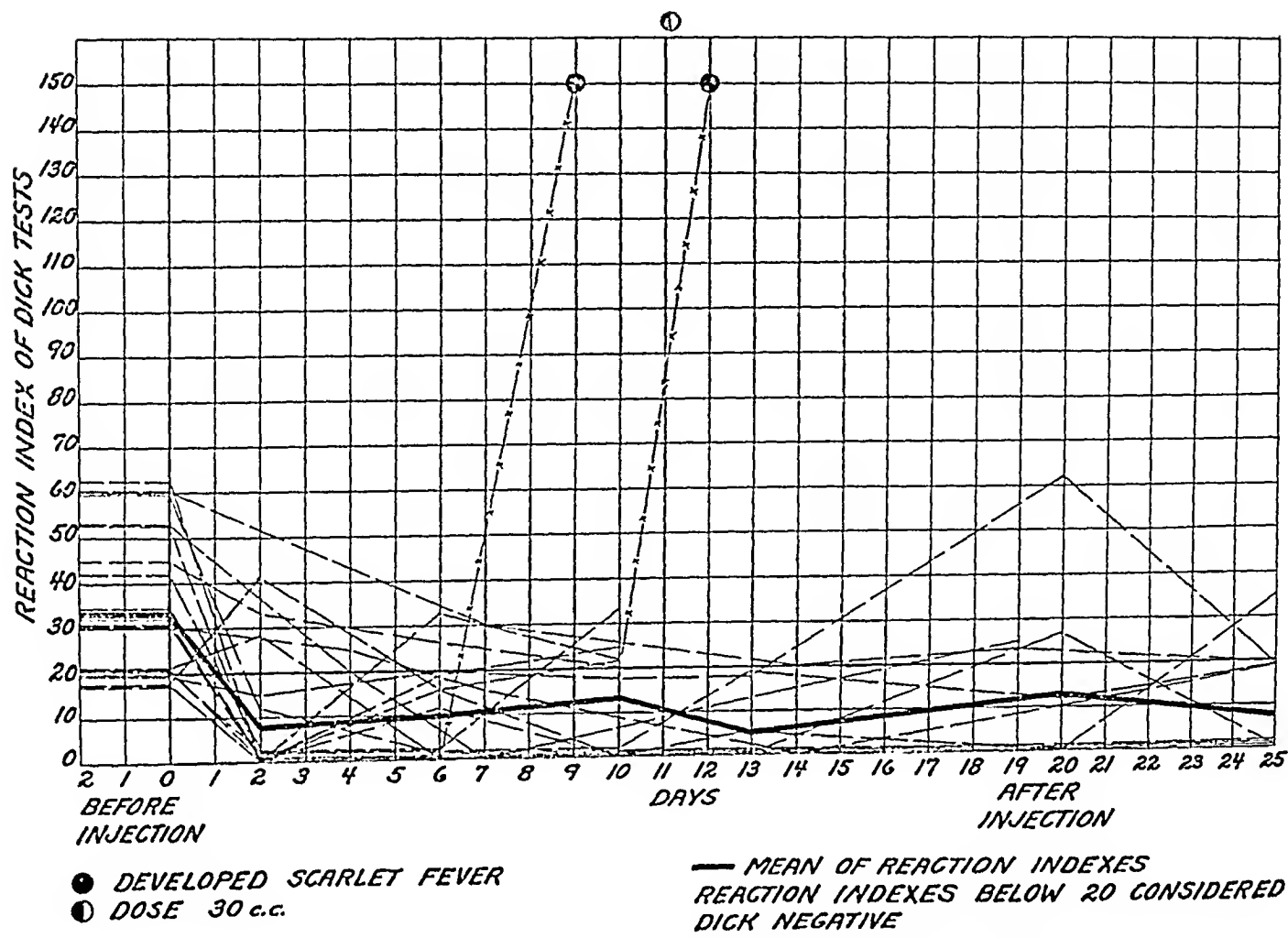


FIG. 2. EFFECT OF POOLED ADULT SERUM ON THE DICK TEST—17 CASES

and physicians are often unwilling to lose the 24 hours which is necessary for the performance of the test. As will be seen in Table I only eight of the four hundred and fifty-three persons (1.8 per

cent) injected developed scarlet fever. The data concerning these eight cases follow.

TABLE I  
*Prophylaxis against scarlet fever in 500 cases*

	Convalescent serum		Pooled adult serum	
	No disease	Clinical cases	No disease	Clinical cases
Home exposure . . . . .	342*	8†	37‡	2§
Hospital exposure . . . . .	103	0	8	0

\* Dick test known to be positive before injection in 111 cases.  
† Dick test known to be positive before injection in 4 cases.  
‡ Dick test known to be positive before injection in 33 cases.  
§ Dick test known to be positive before injection in 2 cases.  
|| Dick test known to be positive before injection in 78 cases.

Case 1. C. W., female, age 3, was given 15 cc. of convalescent scarlet fever serum on December 8, 1934, after an intimate exposure for one day to a brother J. W. who developed clinical scarlet fever on December 7, 1934. J. W. was kept at home, but isolated from the rest of the family. On December 17, 1934, a second brother, P. W., developed what may have been a subclinical case of scarlet fever which may have reexposed C. W. On December 26, 1934, nineteen days following her initial exposure, C. W. developed typical mild scarlet fever. Her temperature never rose above 101° F.

Case 2. R. D., male, age 15 (Fig. 1), was given 15 cc. of convalescent scarlet fever serum on February 15, 1936. This boy was a student in a boarding school of one hundred and sixty-seven individuals, where there had been fourteen cases of scarlet fever in the preceding five weeks. One hundred and thirty-one of the one hundred and sixty-seven members of the school family had throat cultures positive for the hemolytic streptococcus on February 13, 1936, and exposure in this case was probably intimate and prolonged. Ten days following the injection of the convalescent serum, R. D. developed a

marked rash, typical of scarlet fever, which lasted for one day. His temperature remained below 100° F. at all times, and he made a rapid and uneventful recovery.

*Case 3.* B. O'B., female, age 30 (Fig. 1), was given 15 cc. of convalescent scarlet fever serum on February 15, 1936. This individual was a secretary in the same school mentioned in Case 2, and exposure was likewise presumed to be long and intimate. On February 26, 1936, eleven days following the injection of the serum, she developed a mild case of scarlet fever with a moderate rash, a very sore throat, and a temperature of 101° F. She was given 30 cc. of convalescent scarlet fever serum, and her temperature reached normal in about 36 hours from the time the serum was given.

*Case 4.* H. T., female, age 8, was given 15 cc. of convalescent scarlet fever serum on February 10, 1936. She was intimately exposed to a sister who developed scarlet fever on February 9, 1936, and remained in the same house with her sister during the latter's illness. On February 23, 1936, fourteen days after onset of her sister's illness, and thirteen days after the injection of the serum, H. T. developed a very mild case of scarlet fever. She was given 30 cc. of convalescent serum at that time, and her temperature was normal by the next day.

*Case 5.* D. T., female, age 10, was a sister of Case 4 and was exposed under similar circumstances. She was given 15 cc. of convalescent scarlet fever serum on February 10, 1936, and developed a barely distinguishable case of scarlet fever on February 30, 1936, twenty-one days after her initial exposure and twenty days after the injection of the serum. The course of her disease was afebrile throughout.

*Case 6.* M. B., female, age 2, was given 5 cc. of convalescent scarlet fever serum, considered an inadequate dose, on February 24, 1936. She was intimately exposed for three days to a sister who developed scarlet fever on February 22, 1936. The sister was isolated from the other children on the third day of her clinical disease, but remained in the same house. M. B. developed typical moderately severe scarlet fever with a temperature at the onset of 103° F., on March 12, 1936, nineteen days after her initial exposure and seventeen days after the injection of the serum. Her temperature dropped to normal within twenty-four hours following the injection of 30 cc. of convalescent scarlet fever serum.

*Cases 7 and 8.* M. B. and J. B., brothers, age 9 and 10, were first exposed to a brother who developed scarlet fever on October 17th, 1936. They were isolated from this brother, but remained in the same house. On October 19th, they were given lyophile serum, M. B. receiving a mixture consisting of 10 cc. of convalescent scarlet fever serum and 10 cc. of pooled adult serum, and J. B. receiving 20 cc. of convalescent scarlet fever serum. M. B. developed mild scarlet fever on October 22d, and J. B. developed a mild case of the disease on October 24th, five and seven days respectively following their initial exposure, and three and five days after the injection of the serum. Both cases were complicated by cervical adenitis.

It is of interest that all eight of the cases just described remained either in the same house or in the same boarding school with scarlet fever cases from the time of their initial exposure until they developed the disease. Six of the eight cases developed scarlet fever between the tenth and twentieth day following the injection of convalescent serum. In view of these findings and in view of the fact that quantitative reversal of the Dick test following the injection of convalescent serum remains for only ten or twelve days, it would appear wise wherever such intimate contact occurs to administer serum every ten days until the causative organisms have disappeared from the throat of the patient with scarlet fever.

Convalescent serum for the treatment of toxic cases of scarlet fever has been employed by a number of investigators beginning with Weisbecker (13), in 1897. Gordon, Bernbaum and Sheffield (14), in 1928, stated that serum from convalescents had been established as a valuable therapeutic measure. They concluded that convalescent serum and antitoxic horse serums are equally effective in reducing fever, duration of skin lesions, complications, and fatality rate. Convalescent serum has the added advantage in that it does not produce the serum sickness which follows in about 33 per cent of the cases treated with horse serum.

Hoyne, Levinson and Thalhimer (11) have recently reported their results with the use of convalescent scarlet fever serum in the treatment of nine hundred and forty-seven patients. They divided their cases into hospital and home groups; in the hospital group good results were recorded in 75 per cent of the cases, fair results in 11 per cent, and questionable or no results in 14 per cent. In the home group good results were recorded in 81 per cent, fair results in 8 per cent, and questionable or no benefit in 11 per cent. Also they reported that septic complications were fewer in treated cases than in a comparable group of untreated cases.

In the present study, seventy-eight cases of scarlet fever have been treated with convalescent scarlet fever serum. Twelve of these individuals had very mild cases of the disease prior to treatment, and probably would not have been treated had not the attending physicians in question been particularly anxious to prevent the occurrence of

any complications. The remaining sixty-six cases could be classified previous to treatment as moderate or severe. The majority of these cases were given a single injection of convalescent serum, the dose varying from 30 to 60 cc. In several instances, the initial dose was repeated once, and in one case twice, the largest amount of serum received by any individual case being 120 cc.

It is difficult to determine the value of the serum in the twelve very mild cases except to say that they developed no complications. Of the fifty-six moderately severe and severe cases of scarlet fever studied, forty-eight responded favorably to the convalescent serum. In the majority of these cases the temperature reached normal within about thirty-six hours of the time the serum was given; and within the same time the rash had begun to fade, the toxic manifestations had disappeared, and the patients appeared to be convalescent. Eight cases failed to show any remarkable response to serum therapy.

Complications developing among the sixty-eight treated cases of scarlet fever were few in number and may be listed as follows:

Otitis media .....	4 cases
Sinusitis .....	1 case
Cervical adenitis .....	4 cases

#### *Prophylaxis against measles*

The value of convalescent and immune adult serums in prophylaxis against measles is well recognized.

There is no general agreement concerning the optimum time for drawing serum from persons recently convalescent from measles. Apparently this serum is potent from the time defervescence occurs up to at least a year following the disease. In these studies, blood for convalescent serum was taken only from persons who had had measles within the preceding year, and it appeared preferable to bleed them in the first three or four months following their illness.

In the analysis of the present results the cases are tabulated as completely protected, attenuated, or receiving no apparent protection. The disease was considered attenuated if the temperature did not rise above 101° F. at any time, and did not last for more than two days as a maximum. Any case of greater severity was listed as receiving no protection.

When the difference in dosage is omitted from consideration very similar results were obtained with convalescent measles and pooled normal adult serums.

As observed by other workers, attenuation of the disease is preferable to complete protection except for very delicate children, infants, and certain hospitalized children.

The results recorded agree in general with those of other workers, i.e., pooled adult serum is quite as effective as convalescent serum. However, the concentration possible with lyophile serum makes the size of the dose comparatively unimportant; for this reason in the present studies less of the more costly and scarce convalescent serum has been used and more of the pooled adult serum which can be had in any quantity desired at a relatively low cost.

The majority of workers feel that little effect is to be obtained when serum is injected later than the seventh day following the initial exposure. Four of the seven cases in the series who developed typical unmodified measles received their serum on the seventh day following their first exposure. In contradistinction to this, several cases receiving injections later than the seventh day appeared to be protected. Final conclusions may not be drawn, but on the whole, our results, together with those of other investigators, show that late injections provide less protection. Some workers, including Debré and Ravina (15), employ a standard dosage, giving the injection early in the incubation stage if they desire complete protection, and later if modification only is desired. This method appears to be a better means of regulating the severity of the attack than does the modification of the dosage. However, it is impossible in many instances to select the proper date for injection.

It is generally conceded in measles also that pooled serum is better without particular regard to the time that the donor may have had measles. In three children, not included in the cases tabulated in Table II, typical measles developed following the injection of 2.4 cc. per year of age of serum drawn from a single adult donor.

The results with the use of convalescent and pooled adult serum in prophylaxis against measles can be seen from Table II. None of the cases re-

TABLE II

*Prophylaxis against measles in 410 cases*

	Convalescent serum			Pooled adult serum		
	No disease	Attenuated	Clinical cases	No disease	Attenuated	Clinical cases
Home exposure. . . .	40	30	5	94	63	2
Hospital exposure. . .	37	6	3	116	10	4*

\* Dose—2.5 cc. per year of age injected on 7th day of continuous intimate exposure.

ceiving either type of serum suffered any sequelae. The results compare favorably with those reported by other workers concerning the use of fresh serum or placental extract (16).

Although several workers have published reports on the treatment of measles with convalescent serum, the use of the lyophile convalescent serum therapeutically has only been attempted in a few cases and no conclusions may be drawn. Reports concerning the use of convalescent serum for treatment have not been encouraging.

*Prophylaxis and treatment of mumps*

Alfred Hess (17) in 1915 first reported the use of convalescent serum for prophylaxis in mumps. Skrotskiy (18) reported the intramuscular injection of from 5 to 15 cc. of convalescent serum in one hundred and seventy-nine children exposed to mumps. In this group two mild cases of mumps developed, and the remainder appeared to be completely protected. Cambessédès (19) in 1933 reported the successful use of convalescent serum in prophylaxis and also considered that he used this serum to good advantage in the treatment of the disease, reducing by this means the incidence of orchitis. Barenberg and Ostroff (20) reported the use of adult whole blood as well as convalescent whole blood in prophylaxis against mumps, and found that 15 per cent of those injected developed the disease compared with 39 per cent in a control group. Kereszturi, Hauptman and Park (21) were unable to draw any conclusions from their study.

Convalescent and pooled normal adult serum have been used in the present studies (Table III). Nine cases developed among one hundred and forty exposed individuals, six in the group receiving convalescent serum, and three in the

TABLE III

*Prophylaxis against mumps in 140 cases*

	Convalescent serum		Pooled adult serum	
	No disease	Clinical cases	No disease	Clinical cases
Home exposure. . . . .	63	5	8	1
Hospital exposure. . . . .	15	1	45	2

smaller group receiving pooled adult serum. Two of these children developed mumps without any attenuation, twenty-five and thirty-five days respectively, following the administration of presumably adequate doses of convalescent serum. In another child mumps developed twenty-four days following the administration of 20 cc. of pooled adult serum. In these cases, the possibility of re-exposure was excluded. The virus of mumps may have remained quiescent in the presence of protective antibodies, only to become active again when these antibodies became exhausted. Three cases developed mumps on the fifth, seventh and eighth day, respectively, following the administration of serum, and in these cases it is felt that serum was given too late in the course of the incubation period to have been of value. One case, 27 years of age, developed typical mumps seventeen days following a prophylactic injection of only 10 cc. of pooled adult serum. There is no satisfactory explanation for the development of the disease in the other two non-protected individuals.

The majority of cases treated at home were thoroughly exposed to proven cases of mumps. Thirty-two cases reported in the hospital group were exposed to a resident physician who, after contracting the disease, with visible parotid swelling, examined every nose and throat on his ward. None of these thirty-two individuals developed mumps.

No data were available concerning the optimum time to draw serum from persons convalescent from mumps. For this reason six months was arbitrarily taken as the maximum time that should have elapsed from the onset of the disease to the withdrawal of blood. Further data will be necessary before definite conclusions can be drawn as to the optimum time.

Like chickenpox, mumps in children is consid-

ered to be a rather innocuous disease and one which is best contracted before puberty ushers in the hazard of a complicating orchitis or oophoritis. It is the fear of these complications which caused many adults to request an injection of serum for themselves following exposure to mumps in their children. These results suggest that convalescent and pooled adult serums may be effective in the prevention of mumps in exposed individuals.

A single case<sup>4</sup> of severe orchitis in mumps was treated with 50 cc. of convalescent mumps serum on three successive days, beginning with the fifth day of the orchitis. On the third day of treatment the temperature dropped to normal by crisis, and the boy made an uneventful recovery. It cannot be determined whether or not the serum was the deciding factor in this case. From reports in the literature convalescent mumps serum would appear to be a valuable therapeutic measure in this type of orchitis.

#### *Prophylaxis against chickenpox*

The successful use of convalescent chickenpox serum for prophylaxis has been reported by Blackfan, Peterson, and Conroy (22), Mitchell and Ravenel (23), Gordon and Meader (24), and Lewis and Barenberg (25) and by several others. Chickenpox in children is considered a fairly innocuous disease, but is a scourge once it has established itself in a pediatric ward, and for this reason it is in the hospital that prophylaxis is most often sought. The majority of cases reported here are taken from hospital practice where the expected case rate of chickenpox is much lower than that generally found in private practice, although higher than that of mumps and scarlet fever. In the experience of Gordon and Meader (24), 68 per cent of susceptible children exposed to chickenpox in a hospital and not treated contracted the disease.

Gordon and Meader have pointed out that the protective properties of convalescent varicella serum are markedly diminished after three months following defervescence. For this reason the attempt was made to collect the serum within this period. Pooled adult serum was employed in a number of cases and while complete protection

was secured in a moderate percentage of those treated, pooled adult serum did not appear to be as effective in the prophylaxis of chickenpox as in that of measles. Lewis and Barenberg (25) employed a dosage of 30 cc. of adult whole blood in an attempt to protect five individuals from chickenpox, but all five contracted the disease in mild form. With adult serum, however, they apparently protected eight cases with a dose of 40 cc. Most of the published reports deal with the use of convalescent serum.

TABLE IV  
*Prophylaxis against chickenpox in 157 cases*

	Convalescent serum		Pooled adult serum	
	No disease	Clinical cases	No disease	Clinical cases
Home exposure . . . . .	4	7	15	3
Hospital exposure . . . . .	42	1	71	14*

\* 6 of these cases given an inadequate dose of 7.5 cc.

The results in one hundred and fifty-seven cases may be seen in Table IV. It may be noted that a number of failures occurred with relatively small doses of pooled adult serum. Several children receiving what were considered adequate doses of convalescent serum developed the disease without any evidence of attenuation. Three infants in private practice given 10 cc. of convalescent serum within the first three or four days following a heavy exposure to members of their own families, developed a very mild form of chickenpox consisting of 4 to 10 typical lesions but without any elevation in temperature. These results, although in general satisfactory, are not as conclusive as those recorded above in scarlet fever and measles.

#### *The treatment of erysipelas*

The use of convalescent erysipelas serum for the treatment of this disease was first described by Fornaca (26) in 1905; Jordan and Dustin (27), and several others have more recently described the use of this serum with favorable results.

Three severe cases of erysipelas, two facial and one of the leg, have been treated with lyophile convalescent erysipelas serum. These cases made

<sup>4</sup> We are indebted to Dr. John A. Young of Newport, R. I., for the data on this case.



good recoveries, and injection of the serum was followed by a fall in temperature, reduction in toxemia, and fading of the rash. These patients received from two to four injections of serum, each amount injected varying from 30 to 60 cc. No other special therapy such as x-ray, ultra-violet light, or horse serum, was employed in the treatment of these cases.

In view of the fact that immunity to the hemolytic streptococcus of erysipelas is believed to disappear rapidly in certain cases following recovery from the disease (Birkhaug (28)), it is suggested that blood for convalescent serum be drawn when feasible within six weeks after defervescence.

#### *Prophylaxis and treatment of whooping cough*

Bradford (29) has recently reviewed the literature concerning the use of convalescent and immune adult whole blood and serum in the prevention and treatment of whooping cough. The general opinion seems to be that these serums may be effective in the prevention of the disease, although the reports concerning the results in treatment are not conclusive.

Pooled adult serum has been used in these studies in prophylaxis against whooping cough in one small group of twelve infants between the ages of one and seven months. These children were exposed in a ward to a frank case of pertussis who was kept on the ward for two weeks following the onset of the disease, and subsequently died of pertussis bronchopneumonia. Serum was injected on the twelfth day following the initial exposure, eight of the infants receiving 10 cc. of serum and the remaining four 20 cc. None of these infants developed whooping cough. One child of 10 years was given 20 cc. of pooled adult serum following a two day exposure to a sister with the disease. This child also did not develop whooping cough.

Jundell (30) in 1933 reported the use of blood from individuals who had recently had an injection of fresh whooping cough vaccine in the treatment of five cases of whooping cough. It was his feeling that these cases responded well to the blood. For the past ten months we have been injecting several healthy adults with the routine course of Sauer's whooping cough vaccine, giving

one full course of vaccine every four months. At the end of the first four months these donors have been bled, and the bleedings have been repeated at varying intervals thereafter. All of the donors give a history of having had whooping cough in childhood. A good opportunity for testing the serum from the donors has not as yet arisen. It has been given to twelve children intimately and continuously exposed to siblings with whooping cough. Previous attacks of whooping cough could be definitely excluded in these cases. Six of these children never developed the disease. Three developed a cough which lasted for from ten days to two weeks, but which was not associated with a characteristic whoop. The remaining three children developed typical but mild whooping cough. Fifteen cases of whooping cough have been treated with this type of serum after the onset of the disease. Five appeared to be much improved after the injections, but the value of the serum in the remaining ten cases was questionable.

No conclusions may be drawn at present concerning the use of "hyperimmune" human serum in whooping cough. Results so far indicate that it may be of value for passive protection against the disease. The benefit to be derived from the serum once the disease has become active is uncertain.

#### *Prophylaxis against German measles*

German measles is such an innocuous disease that it might almost seem unwise to make any attempt to prevent its occurrence. In spite of this fact, there are times when it is desirable to prevent its spread in a hospital ward. Also there are occasions when for various reasons it would be inconvenient and sometimes even serious for a child in a private home to develop the disease. Pooled adult lyophile serum has been used in one outbreak of German measles in the ward on which occasion ten infants between the age of 6 and 10 months and one infant of 4 months were each injected with 10 cc. of this serum on the day that a typical case of German measles developed in the ward. Two additional cases developed the following two days, and all three cases remained on the ward throughout their disease. Of the eleven children receiving serum, one de-



veloped the disease twelve days after the initial exposure. The rest remained well.

Two infants of 18 months were each given 10 cc. of pooled adult serum three and five days respectively following an intimate exposure at home. Neither of these infants developed German measles.

### *The treatment of acute hemolytic streptococcal infections*

Thalhimer and Levinson (31) have recently reported on the use of pooled convalescent scarlet fever serum in the treatment of "diverse streptococcic infections" such as cervical adenitis following a streptococcic sore throat, "sepsis" and septic complications, acute streptococcal pharyngitis, purulent otitis media, streptococcic pneumonia, etc. Their results appeared to be excellent in 11 per cent of the cases studied, good in 44 per cent, doubtful in 17 per cent and without benefit in 28 per cent.

A relatively small series of cases of hemolytic streptococcal infections has been treated with pooled lyophile convalescent scarlet fever serum, and while no final conclusions may be drawn from these studies, the results obtained in a number of instances have suggested considerable benefit from this therapy.

Six cases of acute hemolytic streptococcal laryngitis have been treated with from 40 to 120 cc. of lyophile convalescent scarlet fever serum. Three of these cases appeared to respond rather dramatically with a rapid fall in temperature, reduction in toxemia, and diminution in the throat symptoms. The other three cases recovered, but the reduction in fever and toxemia following the injection of serum was not striking.

Three cases of hemolytic streptococcal meningitis have been treated by intraspinal injections of convalescent scarlet fever lyophile serum. Two cases appeared to be benefited for a short time as indicated by a temporary but marked reduction in the number of cells in the spinal fluid, and by a temporary disappearance of the organisms following the injections. These two cases finally died. The third case<sup>5</sup> made a complete recovery. In this case diagnosis was made and a

<sup>5</sup> We are indebted to Dr. John P. Scott and Dr. Samuel X. Radbill for the data on this case.

bilateral mastoidectomy performed on what was presumably the fifth day of the disease. The day following the operation a blood transfusion was given and on the next three successive days 30 cc. of convalescent scarlet fever serum was given intraspinal as well as 30 cc. of serum intramuscularly. Spinal fluid culture was positive on the fourth day following operation but the plates only showed 2 to 3 colonies, whereas the spinal fluid immediately preceding and after operation showed many organisms on direct smear as well as on culture. By the fifth day after operation and the third day after the initiation of serum therapy the temperature was normal, and remained normal thereafter. Thirteen days after operation, and eleven days after the first dose of serum the child was convalescent, and the spinal fluid was clear, negative to culture, and contained only 42 cells per cubic millimeter.

Seven cases of severe hemolytic streptococcal sore throat have been treated with convalescent lyophile scarlet fever serum, and all of these cases appeared to be benefited. Two cases of acute mastoiditis with suppurative otitis, of hemolytic streptococcal origin, were treated with the same type of serum and recovered, but the value of the serum in these cases was masked by the repeated blood transfusions which were given in addition to the serum.

One case of puerperal sepsis with an associated hemolytic streptococcal septicemia was treated with one 300 cc. blood transfusion and three injections of 40 cc. of convalescent scarlet fever serum given at twelve hour intervals. This individual made a rapid and uneventful recovery.

One case of hemolytic streptococcal septicemia of obscure origin died following treatment with convalescent scarlet fever serum, repeated blood transfusions, and antistreptococcal horse serum.

### *Reactions*

Moderately severe reactions have occurred in only five instances in over fifteen hundred injections of lyophile serum. These children all developed a temperature of about 104° F., local tenderness and swelling, and marked malaise, all symptoms starting within the first four to six hours following the injections and lasting 36 to 48 hours. One of these children developed a pur-

puric rash which disappeared within 12 hours. These five reactions followed the administration of serum which had been prepared during the early part of the work, and which was markedly hemolyzed. Whether or not hemolysis had anything to do with these reactions we cannot be sure. It seems significant, however, that from the time the serum was produced with very few hemolyzed cells this severe type of reaction was no longer noted.

Twelve children developed local pain and tenderness with an elevation in temperature of from one to three degrees with slight general malaise such as follows typhoid vaccination. Seven of these children received serum from two small lots prepared early in the studies. This type of reaction usually disappeared overnight.

Lyophile serum when injected intramuscularly causes no greater soreness than the same amount of fresh human serum which has not been processed. This soreness rarely lasts for more than a day, and in the majority of instances there have been no complaints at all.

The amount of serum and the concentration of the serum seemed to bear little relation to the severity of the reactions. The most severe reactions followed doses of about 10 cc. or less of serum. As much as 80 cc. of serum (doubly concentrated) has been given at one injection without any reaction whatever other than muscle tenderness lasting overnight. Abscess formation or tissue necrosis has never followed the injection of lyophile serum.

Serum sickness, such as frequently follows the injection of horse serum, has never been noted in any of these cases. Such reactions have been reported in a few cases following the injection of liquid human serum (32).

It is well known that an allergic reaction may occur following the injection of serum from a donor who has recently eaten food to which the recipient is markedly sensitive. For this reason the donors are bled, whenever possible, in the morning when they have had no heavy meal for some hours.

#### SUMMARY

The use of human serums preserved by the lyophile process in the prophylaxis of scarlet fever, measles, mumps, chickenpox, whooping cough,

and German measles, and in the treatment of scarlet fever, mumps, erysipelas, whooping cough, and acute hemolytic streptococcic infections is described. Particular emphasis has been laid on the use of serum from large pools of normal healthy adult blood in the prophylaxis of the more common contagious diseases.

The results obtained compare favorably with those reported by other investigators who have employed fresh serums or serums preserved in the liquid state. Certain distinctive advantages of lyophile serums (2) are indicated and illustrated with case material.

We are indebted to Miss Janet Armstrong for assistance in the collection of blood, to Dr. Arthur D. Waltz and Miss Belita de Ayala of the Children's Hospital Laboratory for the Kahn reactions, and to Dr. L. J. Wenger for assistance in the performance and reading of the Dick tests involved in the preparation of Figures 1 and 2. The lyophile serum for the past two years has been processed at the Mulford Laboratories of Sharp and Dohme. This work has been aided by a generous grant from the Board of Managers of the Abington Memorial Hospital, and by the interest and foresight of its Medical Director, Dr. Harry B. Wilmer.

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# THE RESPONSE OF NORMAL INDIVIDUALS AND PATIENTS WITH DIABETES INSIPIDUS TO THE INGESTION OF WATER<sup>1</sup>

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The physiological adjustments which follow the ingestion of excessive amounts of water and culminate in the familiar water diuresis are by no means understood. Demonstrable changes in the blood attributable to the absorption of water from the intestinal tract are so trifling in comparison with the enormous fluctuations in urine volume as to militate against suggestion that hydremia is *per se* the immediate stimulus to water release. Indeed, it has been difficult to demonstrate any consistent blood dilution after the absorption of even huge amounts of water, the more so because reduced concentrations of the colloidal non-diffusible constituents of blood do not necessarily imply an increment of circulating water. Haldane and Priestley (16), Adolph (1) and Veil (39) were unable to reduce the concentration of hemoglobin significantly by forcing fluids, and Davis (10), Dresel and Leitner (11), Fee (13), Verney (40) and Smirk (34, 36) observed no correlation between this value and the intensity of experimental polyuria; the large reductions reported by Marx (23, 25) seem decidedly beyond the usual experience. Macallum and Benson (21) and Siebeck (33) noted no drop in the erythrocyte count following the ingestion of water but Daniel and Höglér (9) reported dilutions to 12 per cent. In the experiments of Engel and Scharl (12), Priestley (28) and Rioch (30) the refractive index of serum did not change significantly throughout the course of water diuresis, and Strauss and Chajes (37), Brunn (6) and Daniel and Höglér (9) reported only minimal declines; the dilutions of 10 to 20 per cent found in infants by Bakwin (2) and in decerebrate dogs by Bayliss and Fee (3) are unique. Variations in the concentrations of plasma or serum proteins are also so slight as to be of doubtful importance (Brunn (6), Veil (39), Dresel and Leitner (11),

Rioch (30, 31), Fremont-Smith, Putnam and Cobb (14), Smirk (34, 36)). Jones (18) found but the slightest drop in blood specific gravity after drinking water; Verney (40) no change in plasma colloidal osmotic pressure; Daniel and Höglér (9) and Rioch (30) no important change in serum viscosity; Davis (10), Veil (39) and Fremont-Smith et al. (14) very slight reductions in freezing point depression; Margaria (22) a slight increase in the vapor pressure of blood. The blood volume studies of Marx and Mohr (25) and Dresel and Leitner (11) are contradictory and difficult to evaluate. A somewhat greater consistency has been obtained by the gravimetric estimation of total solids: the reports of Blix (4), Rominger (32), Priestley (29), Davis (10), Marx (24), Brahn and Bielschowsky (5), Rioch (31), Fremont-Smith et al. (14) and Smirk (34, 36) indicate that a drop of 1 to 2 per cent may be expected to accompany water diuresis. These data seem to show that hydremia, if present, must be extremely slight. The extreme values reported by Greene and Rowntree (15), Underhill and Sallick (38) and Chanutin, Smith and Mendel (8) were obtained from animals intoxicated with water.

In contrast are the relatively consistent reports concerning variations in blood electrolytes. Although Bayliss and Fee (3) found no change in the serum electrical conductivity of decerebrate dogs following water administration in nearly half of their experiments, Wilson (41), Priestley (29) and Rioch (30, 31) have described consistent reductions of 3 to 6 per cent, and these are for the most part substantiated by the chloride determinations of Brunn (6), Priestley (29), Marx (23), Dresel and Leitner (11), Fremont-Smith et al. (14) and Smirk (36). The exact figures vary somewhat but with the exception of some of Bayliss and Fee's experiments, the drop in electrical conductivity seems to be uniformly greater than the dilution of the nondiffusible blood constituents

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and markedly in excess of any change attributable to diminished serum viscosity. It appears, therefore, that the plasma hypotonia which follows the ingestion of water is due largely to the rapid outward diffusion of salts into the intestinal contents and body tissues (Burns and Visscher (7)) and only in small part to hydremia.

It has been repeatedly asserted that this reduction in total osmotic pressure is responsible for the ensuing diuresis, but a causal relationship is pure assumption. Priestley (29) maintained that the increased diffusion pressure of blood water directly depresses the capacity of the renal tubule to reabsorb water; but Verney and his associates (19), stressing the similarity between the polyurias of water diuresis, the isolated kidney and diabetes insipidus, have emphasized the importance of the time interval that separates the disappearance of the water from the gut and its reappearance in the urine. By a variety of methods, the important fact has been established that the peak of diuresis does not occur until well after the maximum decrease in the total molecular concentration of plasma has been passed (Marx (24), Rioch (30, 31), Dresel and Leitner (11), Fee (13), Verney (40), Bayliss and Fee (3), Heller and Smirk (17), Verney and associates (19), Smirk (34, 35, 36), Newton and Smirk (27)). Rioch, for example, found that the maximum dilution of blood electrolytes preceded maximum diuresis by 15 to 20 minutes; Verney that the maximum water load of the body was attained about 15 minutes before the peak of diuresis; Newton and Smirk that the average diuresis did not begin until 40 minutes after the administration of water, that maximum elimination was reached in 126 minutes, and that a 20 minute lag existed between the maximum water load and the peak of diuresis. It is in terms of the latent period that the essential stimulus to water excretion has been sought.

Klisiecki, Pickford, Rothschild and Verney (19) explain the latent period by postulating that the renal absorption of water is dependent upon an adequate concentration in the blood of a pitressin-like substance, the manufacture of which is regulated by "the concentration of water in the blood and tissues, as signified by their aqueous vapour pressures." The delay in diuresis is therefore due to the time required for blood pi-

tressin to fall below its threshold level in response to the electrolyte dilution created by water ingestion. The polyurias of the perfused isolated kidney, of the piqûre experiments and of diabetes insipidus are likewise attributed to hypopitressinemia and are, of course, unaccompanied by blood dilution. In order to substantiate this theory it becomes necessary to demonstrate that in these latter conditions the delayed renal excretion of water so characteristic of water diuresis is diminished or absent. Verney himself (19) has published preliminary observations on one case of diabetes insipidus which seem to indicate that this is in fact true, and it was with the object of confirming his findings that the following work was undertaken.

#### METHODS

Throughout these relatively acute experiments the subjects have voided at 10 to 20 minute intervals and have drunk frequently in amounts approximating their normal requirements. Except as stated below in regard to some of the observations on diabetes insipidus, it is believed that all subjects were normally hydrated. After a satisfactorily steady flow of urine was established a large volume of water (1200 cc. for the normals, 2000 cc. for the patients) was drunk within 5 minutes, and the urine collections were continued until well into the ebb of diuresis. Blood samples were taken from the basilic vein, usually with no stasis whatever; occasionally momentary pressure was necessary, but control estimations showed that transient mild pressure did not alter the results. Blood was drawn into a dry syringe and the serum separated by centrifugation as soon as clotting was complete. No attempt was made to prevent loss of  $\text{CO}_2$ . Total solids were determined by drying approximately 1 cc. samples to constant weight at  $105^\circ \text{C}$ . The average deviation from the mean of 6 control estimations from the same sample of serum was  $\pm 1.65$  per cent. Serum proteins were determined refractometrically. The specific conductivity was determined by the usual bridge method, using a Leeds and Northrup 1000-cycle generator. The cell was immersed in a thermostatically controlled water bath at  $25 \pm 0.002^\circ \text{C}$ . The cell used requires about 0.4 cc.; its cell constant is 8.16 at  $25^\circ \text{C}$ ., where cell constant is defined as observed resistance divided by

specific resistance. Determinations were reproducible to 0.05 per cent.

### RESULTS

Eight control observations have been made on 3 normal subjects. In all 8 the electrical conductivity of the serum was followed, and in 5, the total blood solids were also determined; in the remaining 3 experiments serum proteins were estimated refractometrically. After the ingestion of a large volume of water the average reduction in specific conductivity for the entire series was 2.7 per cent, the smallest being 1.75 per cent and the greatest 4.9 per cent. The 5 curves illustrating changes in concentration of total solids showed marked inconsistencies. Not much importance is attached to them, as the fluctuations were not often significantly beyond the apparent limit of analytical error. Their general direction, however, is negative and we believe they indicate slight hydremia, since, in 4, the maximum deflection averaged  $-3$  per cent while 1 showed a maximum increase of nearly 2 per cent. The re-

fractometric readings were even more anomalous, varying from  $+8$  per cent to  $-7$  per cent with virtually no average deviation from normal.

Figure 1 shows a typical experiment on each of 3 subjects, 1 with estimations of serum protein and 2 with estimations of total solids. Attention is called to the very definite time interval that separates the point of maximum electrolyte dilution in the blood from the maximum rate of urine formation. In 7 acceptable experiments the apparent intervals were 45, 20, 40, 40, 40, 40 and 35 minutes, an average of 37 minutes.

Attention was then turned to diabetes insipidus with the expectation that, Verney's theory being correct, this time-lag would be much diminished. To our surprise we were unable to produce anything which resembled a flood diuresis, the urine flow remaining relatively constant under varying conditions of fluid intake. Figure 2 (Curve A) shows a prolonged experiment on such a patient. The blood changes are somewhat more pronounced than in the average normal; but, in spite of the ingestion of 2000 cc. of water above the

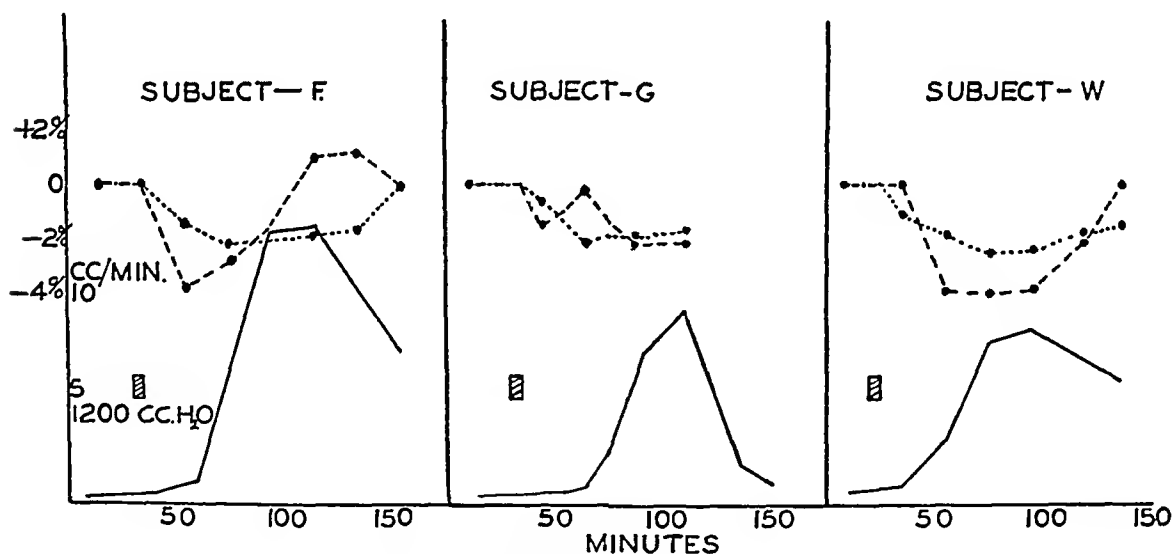


FIG. 1. THE RESPONSE OF NORMAL SUBJECTS TO THE INGESTION OF WATER

●—● = urine flow in cc. per minute. ●...● = serum specific conductivity. ●---● = serum total solids in Subjects F and G, serum refractive index in Subject W. The serum values are plotted as percentage deviations from the pre-ingestion levels. Initial serum specific conductivity:  $F = 0.01227$ ;  $G = 0.01186$ ;  $W = 0.01189$ . Initial serum total solids:  $F = 7.64$  grams per cent;  $G = 8.88$  grams per cent. Initial serum protein:  $W = 7.87$  grams per cent. The shaded rectangles indicate the ingestion of 1200 cc. tap water.

The uniform drop in the electrical conductivity values is in contrast to the irregular and probably unimportant fluctuations in total solids and protein determinations. The time interval separating the maximum decrease in specific conductivity of the serum from the peak of diuresis is to be noted.

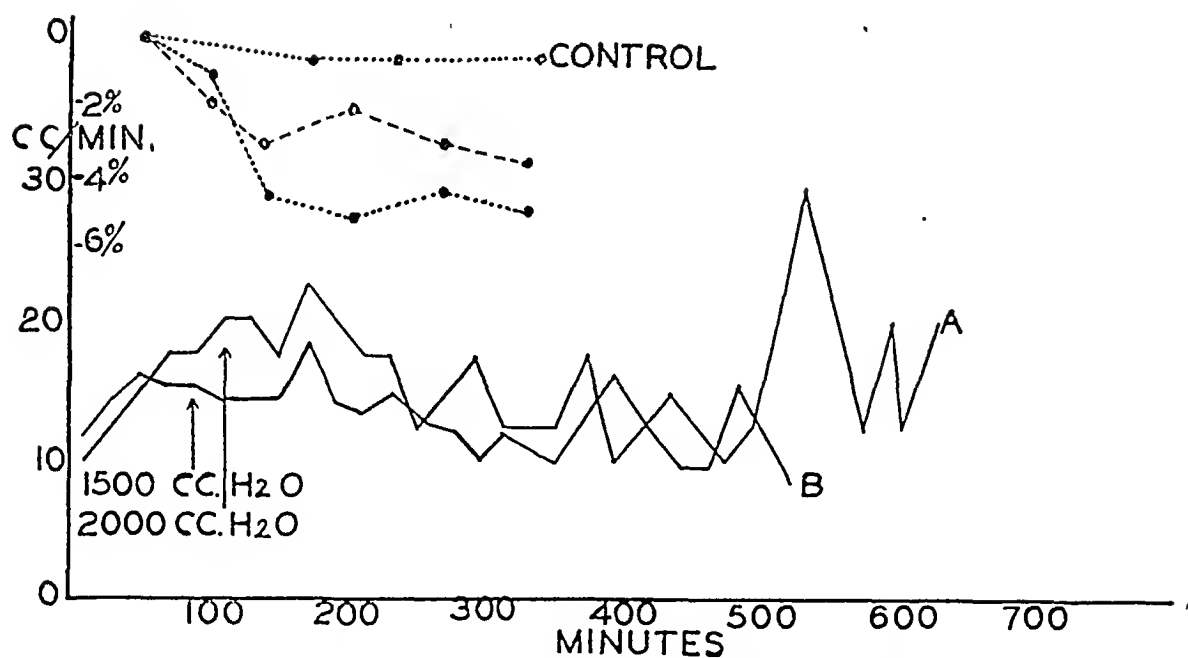


FIG. 2. WATER RETENTION IN DIABETES INSIPIDUS. SUBJECT B

●—● = urine flow in cc. per minute. ●...● = serum specific conductivity (initial value, 0.01116); ●---● = serum total solids expressed as percentage deviation from the pre-ingestion value (initial value, 8.13 grams per cent).

Experiment *A* shows the blood and urine changes accompanying the ingestion of 2000 cc. tap water. Throughout the entire observation period the patient drank 400 cc. water every 20 minutes. In Experiment *B* the basal water intake was 200 cc. every 20 minutes; no blood studies were made. Despite the submaximal excretory rate and the large dilution of blood electrolytes no diuresis was initiated in either case. The control electrical resistance determinations were made at random intervals during a normal unrestricted day.

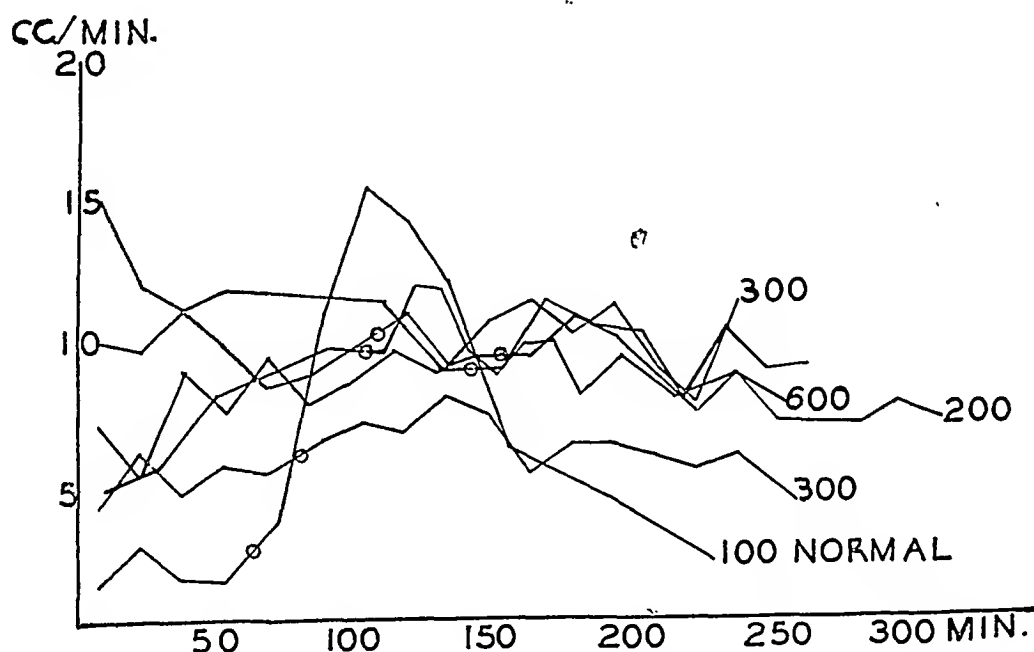


FIG. 3. WATER RETENTION IN A SECOND CASE OF DIABETES INSIPIDUS. SUBJECT D

Urine flow is recorded in cc. per minute. The circles represent the ingestion of 1200 to 2000 cc. tap water, and the figure appended to each curve represents the hourly basal fluid intake maintained throughout the experiment and given at 15 to 20 minute intervals. Despite widely varying basal urine output the patient released extra water very slowly, in comparison with the depicted normal response.

patient's basal intake, no diuresis occurred within 8 hours. Since the basal fluid intake throughout the experiment was 1200 cc. per hour, rather more than he ordinarily consumed, it seemed possible that his kidneys were already responding at a maximal rate and that no increase in urine formation should be expected. However, he had been repeatedly known to excrete more than 20 cc. per minute. Despite the fact that this figure represented a submaximal excretory rate it was decided to repeat the observation when the basal urine flow was considerably below the known capacity of the kidneys. Curve *B* in Figure 2 represents a repetition of this experiment, without blood data, however, done on a basal fluid intake of 600 cc. per hour. Figure 3 represents a series of efforts to induce water diuresis in a second patient with diabetes insipidus. By varying the basal fluid intake the urine flow could within certain limits be controlled, although identical intakes on different days resulted in variable urine outputs. It will be seen that despite widely varying basal urine outputs no curve resembling water diuresis was ever obtained.

#### DISCUSSION

Water diuresis has been studied in diabetes insipidus with the anticipation that the latent period between the point of maximum blood electrolyte dilution after water ingestion and the peak of diuresis would be less than normal, if Verney's theory is correct. No evidence either for or against this hypothesis has been obtained, however, since it proved impossible to influence the rate of urine flow appreciably by the oral administration of water, although the blood changes were normal in kind and somewhat exaggerated in magnitude. We recognize the dangers incurred in speaking of optimal water requirements in diabetes insipidus and the relative futility of attempting to correlate fluid intake and output in this condition, but call attention to the fact that no sharp and significant rise in urine flow was ever induced even though the patients were not thirsty and were excreting urine at rates below the known capacity of their kidneys to do so. If it be argued that the diabetes insipidus patient is polyuric because he lacks pitressin then some accessory mechanism of water control must be

postulated to account for his capacity to retain extra water for a period of at least 8 hours, even in the absence of demonstrable cardiorenal disease. We are therefore unable to confirm Verney's claim that water diuresis is less delayed in diabetes insipidus than in the normal since our patients exhibited no clear-cut renal response to ingested water. On the contrary, it appears that the relative urine volume response of the diabetes insipidus individual to ingested water resembles that of the normal subject to the ingestion of isotonic saline. It is not claimed, of course, that this phenomenon is exhibited by every subject with the disease, for Leschke's patient (20) did excrete an extra 1.5 liters of water in 4 hours. Marx (26), however, has noted that his patients excreted only 20 to 30 per cent of excess water within 4 hours.

#### CONCLUSIONS

1. In confirmation of previous work the oral administration of water to normal individuals produces only a slight hydremia, but a definite dilution of blood electrolytes. After an average lag of about 35 minutes diuresis reaches its height.

2. Oral administration of water to individuals with diabetes insipidus, under conditions which should permit an increase in urine output, results in similar blood changes, but in a small and greatly prolonged renal response.

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# PARATHYROID HYPERPLASIA IN RABBITS PRODUCED BY PARENTERAL PHOSPHATE ADMINISTRATION<sup>1</sup>

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From the observations of Bergstrand (1) and others (2, 3, 4) it has become an established fact that some cases of chronic renal insufficiency are accompanied by enlargement of the parathyroid glands. The condition was well exemplified by a case recently studied at the Massachusetts General Hospital in which chronic glomerular nephritis had existed for more than twenty years, and in which necropsy revealed tremendous enlargement of all parathyroid glands (5).

The question as to the cause of the parathyroid enlargement arises. It was suggested by Albright, Baird, Cope and Bloomberg (6) that the phosphate retention in chronic renal insufficiency might be the determining factor. When the blood phosphate level is raised by intravenous phosphate administration, hypocalcemia and tetany ensue (7). It seemed not unlikely that either hyperphosphatemia or the resulting hypocalcemia might be a stimulus to parathyroid hyperplasia. The present investigations were undertaken to determine the effect of administration of parenteral phosphate on the parathyroid glands of rabbits.

## METHOD

The animal selected was the rabbit because the veins are easily accessible, and the inferior parathyroids are readily identifiable. These rabbits were fed the ordinary laboratory diet of oats, carrots, and hay. Most of the animals had been used from one to several weeks previously for the Friedman modification of the Aschheim-Zondek test (8), but several males and previously unused females were included in the control and experimental groups.

For injection, a buffered solution of sodium phosphate was prepared. The stock solution was made up as follows.

NaH<sub>2</sub>PO<sub>4</sub> ..... 160 grams  
NaOH 2.5 N ..... 410 cc.  
Distilled water up to ..... 850 cc.

Then 43.93 cc. of this concentrated solution was diluted to 500 cc. with distilled water producing a solution of pH 7.3 isotonic with the blood, and containing 25 mgm. of inorganic phosphorus per 10 cc. The solution was prepared in chemically clean glassware and, after autoclaving, was kept in a refrigerator to prevent growth of possible contaminants, especially molds.

In most cases the animals were injected three times a day intravenously, the quantity usually being 10 cc. (= 25 mgm. P) each time. Some of the rabbits were given additional subcutaneous injections in 10 cc. doses. At varying intervals the animals were sacrificed and autopsied. This included the removal and immediate weighing on a micro balance of the inferior parathyroid glands.

TABLE I  
*Experimental group*

Rabbit serial number	Weight	Weight of inferior parathyroids	Number of days injected	Number of injections		
				Intra-venous	Subcu-taneous	Total
	<i>kgm.</i>	<i>mgm.</i>				
902	1.8	22	108	324	215	539
746	2.5	45	80	240	32	272
903	1.9	23	56	168	59	227
835	2.5	35	54	162	53	215
707	1.6	35	49	147	0	147
643	2.55	*	33	99	0	99
829	2.5	25	28	84	5	89
683	2.0	24	22	66	0	66
7	1.85	7	21	63	63	126
1	1.55	9	20	60	60	120
837	1.7	18	17	51	0	51
901	2.15	14	16	48	0	48
602	2.1	*	15	45	0	45
11	1.8	7	8	24	24	48
987	2.1	11	7	21	21	42
991	2.1	13	7	21	21	42
1000	1.9	10	7	21	21	42
738	2.0	25	2	6	0	6
710	2.25	17.5	1	4	0	4
Average 20						

<sup>1</sup> This investigation was made possible by a grant from the Proctor Fund of the Harvard Medical School.

\* Hyperplasia (not weighed).

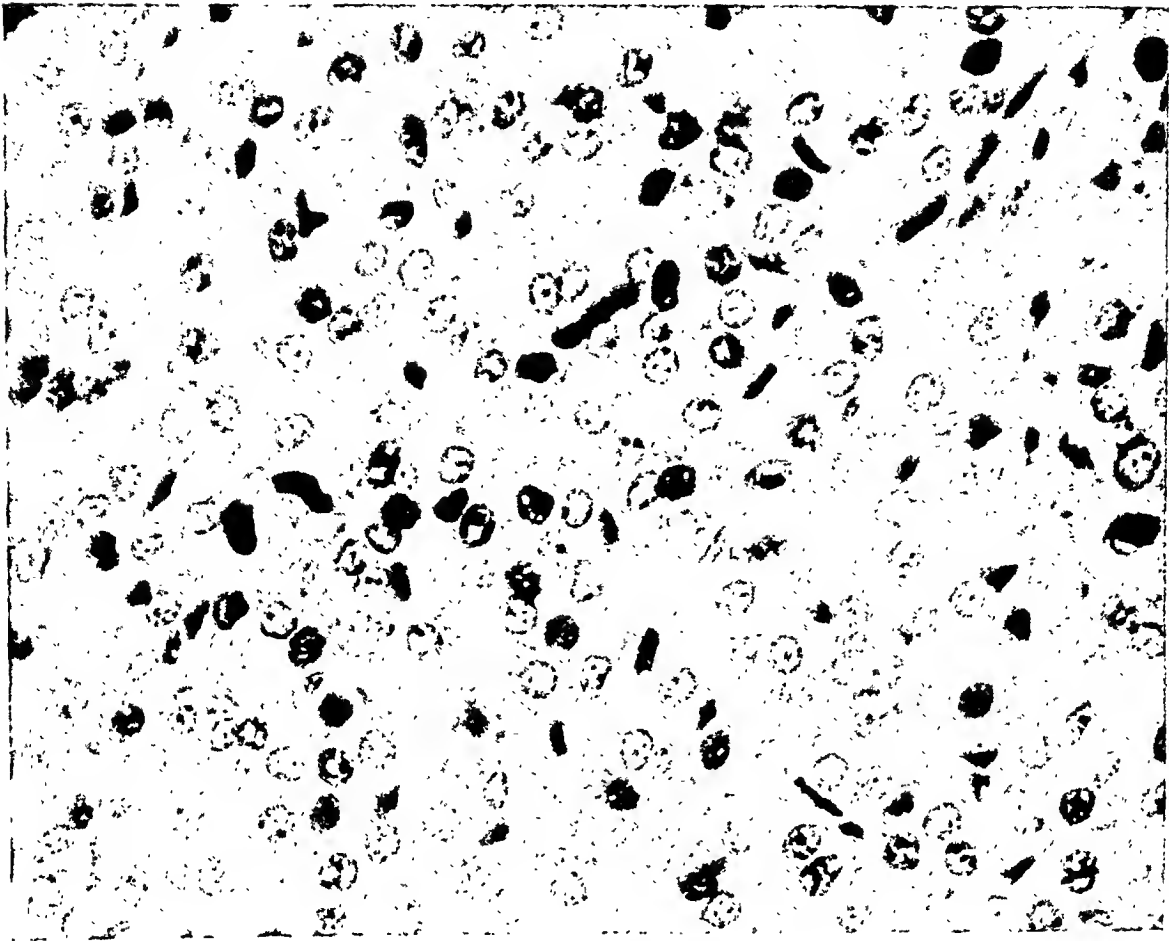


FIG. 2. A PHOTOMICROGRAPH OF THE PARATHYROID GLAND OF A RABBIT INJECTED WITH PHOSPHATE, SHOWING THE COMPACTNESS OF THE CELLULAR STRUCTURE OF THE GLAND. X 400

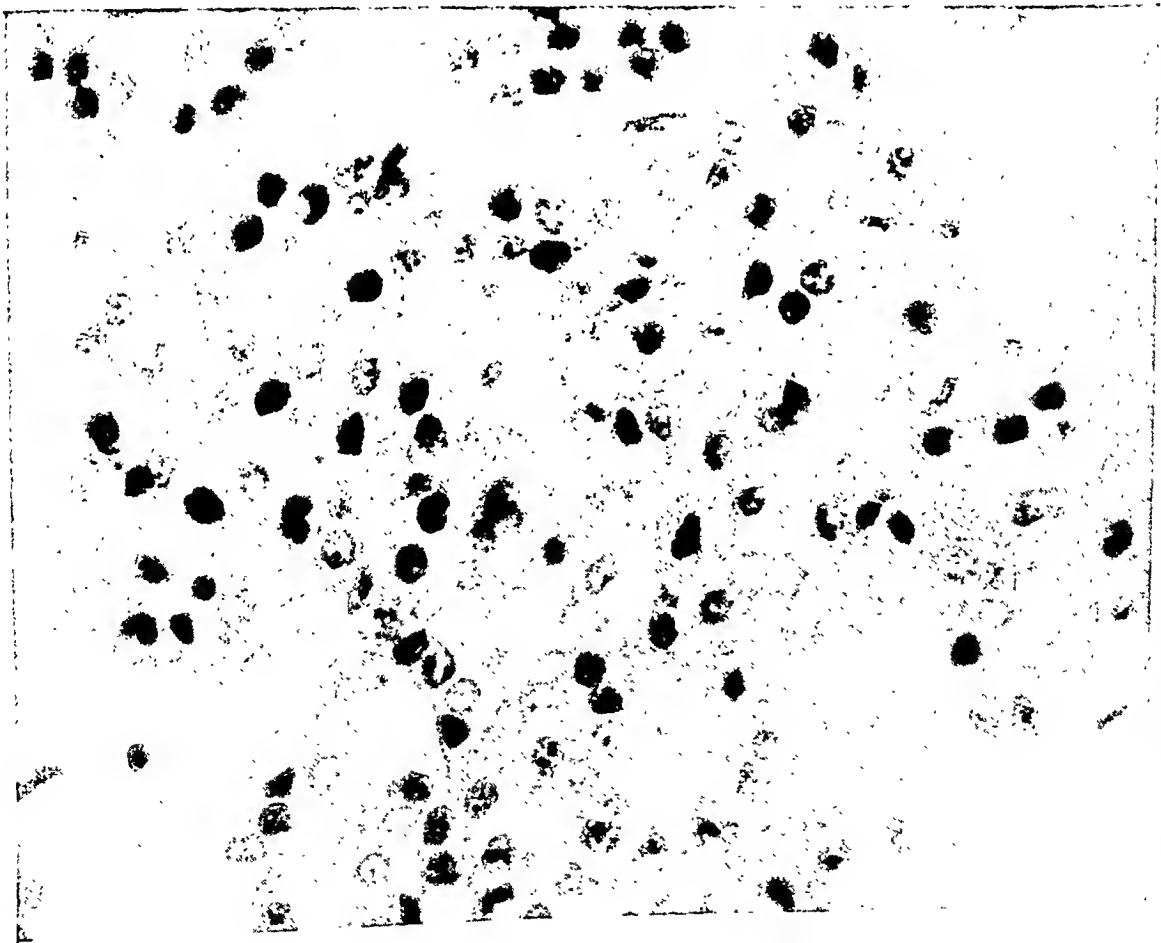


FIG. 1. A PHOTOMICROGRAPH OF A RABBIT'S NORMAL PARATHYROID SHOWING THE LOOSENESS OF THE CELLULAR STRUCTURE OF THE GLAND. X 400

The superior pair were disregarded because they are usually small and are buried within the substance of the thyroid gland.

### RESULTS

Tables I and II indicate the results obtained in the experimental and control groups.

TABLE II  
Control group

Rabbit serial number	Weight	$\frac{\text{Weight of inferior parathyroids}}{\text{Weight of body}}$	Number of days in our animal farm
	kgm.	mgm.	
905	2.8	20	110
978	2.2	10	21+
892	2.1	7	?
C	2.0	8	10+
10	2.0	19	9
907	2.0	7	?
898	2.0	12	?
992	1.95	12	21+
900	1.9	21.4	23+
995	1.85	12	46+
A	1.8	8	30
E	1.75	12.5	?
946	1.75	23	4+
B	1.7	7	60
12	1.7	6	15
D	1.65	12.5	?
883	1.6	17	13+
915	1.45	14.8	3+
837	?	18	2+
Average 13			

There were 19 rabbits in each series which would seem to be enough to permit of valid conclusions. The average weight of the animals was approximately 2 kilograms. Normally there is no correlation between the weight of the animal and the combined weight of the inferior parathyroids.

The glands of the injected group averaged more than 50 per cent heavier than those of the control animals, and this figure would be considerably higher if only those animals injected over three weeks were included. The heaviest glands in the experimental group weighed about twice the maximum observed in the control group.

### HISTOLOGICAL STUDIES

Microscopic studies of the glands leave no doubt as to the effect of the phosphate injections on the parathyroids of the experimental group. The slides of both the control and experimental animals were mixed together and one of us (B. C.) was able to differentiate between the two

groups in 80 per cent of the cases. Difficulty in recognizing hyperplasia was encountered in only a few cases—those injected for 7 to 8 days. The rabbit injected for one day (Number 710) showed definite hyperplasia in three-fourths of the gland; Number 738, injected for two days, showed generalized hyperplasia.

The normal rabbit parathyroid (Figure 1) is composed predominately of chief cells very loosely grouped together. The intervening stroma gives the impression of being slightly edematous and quite vascular. There is no definite architecture although in some places there is a slight tendency to pseudo-acinar arrangement around small blood vessels. The striking feature to keep in mind is the looseness of structure and the relative non-apposition or non-contiguity of cells. In addition, there are occasional single oxyphil cells.

In sharp contrast the parathyroid gland in the experimental group (Figure 2) shows a definite increase in the number of chief cells. The loose architecture has disappeared. The edematous appearing non-cellular areas are filled in with apparently newly formed parathyroid cells. The blood vessel walls, except for the large vessels, have been compressed by this increased cellularity so that they appear as narrow channels, and all tendency to acinar arrangement has been obliterated. The appearance is that of a very densely packed mass of cells. Measurements of the size of the cells show practically no increase over the normal, so the process is one of hyperplasia rather than hypertrophy. A very occasional mitotic figure is seen and almost no oxyphil cells are found.

Microscopic and x-ray examination of the bones shows no difference between the two groups, and there is no evidence of a rachitic process that might produce a secondary parathyroid hyperplasia. The kidneys and other organs appeared normal.

### SUMMARY AND CONCLUSIONS

1. The average weight of the inferior pair of parathyroid glands of 19 control rabbits was 13 mgm.; the corresponding figure for 19 rabbits which had received injections of parenteral phosphate three times daily for 1 to 108 days was 20 mgm.

2. The parathyroid glands of the injected ani-

mals showed definite histological evidence of hyperplasia.

3. The findings support the hypothesis that phosphate retention is the cause of the parathyroid hyperplasia in cases of chronic renal insufficiency; it will require further studies to show whether the hyperphosphatemia causes the hyperplasia directly, or indirectly by producing a hypocalcemia.

Acknowledgment is made of assistance by Dr. Hirsh W. Sulkowitch in calculating the formula for a proper phosphate solution, and to Dr. Tracy B. Mallory for preparation of the photomicrographs.

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# PROGNOSTIC VALUE OF THE PRECIPITIN TEST IN MENINGOCOCCUS MENINGITIS

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The occurrence of soluble specific substance of *N. intracellularis* in the spinal fluid in cases of meningococcus meningitis has been demonstrated by the precipitin reaction, but there is no record of an effort to correlate this reaction with the clinical severity or with the outcome of specific treatment of the disease in man. A review of reports of investigations carried out on the precipitin test is given by Rake (1); subsequently Maegraith (2) published additional data.

An epidemic of meningococcus meningitis in Baltimore<sup>1</sup> provided an opportunity for study of the spinal fluid in 69 cases. Antimeningococcus serum was administered intrathecally at least once in 24 hours until 2 negative cultures were obtained. Where there was an obvious invasion of the blood stream, serum was also used intravenously. In an effort to evaluate the severity of each case, the following determinations were made on the first spinal fluid withdrawn from each patient after admission to the hospital and before serum had been given: the spinal fluid cell count, sugar content, degree of phagocytosis and a rough estimation of the number of organisms present. The precipitin test was used in an attempt to estimate its value in rapid typing of the organisms. Early in the study, it became apparent that the intensity of the precipitin reaction was of prognostic value. It is with this specific problem that the present report will deal.

The technique used for the precipitin test was that of the ring test carried out in tubes of small diameter in which centrifuged spinal fluid is carefully layered on type specific precipitin sera (1). A rough quantitative estimation of the amount of specific soluble substance by the quantity of pre-

cipitate formed at the interface of spinal fluid and serum was found unsatisfactory.<sup>2</sup> The time of appearance of the ring gave a more objective result. Readings were therefore made on the first spinal fluid 10 minutes and 1 hour following the setting up of the test. The sera of all cases showing negative tests at 1 hour were retested after exposure to room temperature for 48 hours and many after incubation at 37° C. for 24 hours. These observations will be published in a separate report (3). Only the 10-minute reading will be discussed here. The cases have been divided into precipitin positive and precipitin negative groups according to the result within this period.

TABLE I  
*Correlation between the time of appearance of the positive  
precipitin test and the final outcome of the case*

Precipitin test, 10 minutes	Fatal cases		Recovered cases	
	Number	Per cent	Number	Per cent
Positive.....	17	77.3	5	22.7
Negative.....	2	4.3	45	95.7

Of the 22 cases showing a positive precipitin test within 10 minutes, 17 or 77 per cent were fatal (Table I). Four of the 5 cases which recovered in this group were more resistant to serum therapy than the majority of cases in the negative precipitin group, 4 days or more being required before the spinal fluid became sterile. One of these showed residual bilateral deafness and another returned 2 months later with a recurrence of meningitis. Forty-seven cases gave negative precipitin tests at the end of 10 minutes. Only two of these, 4 per cent, were fatal, both of them of the fulminating septicemic type with mild

<sup>1</sup> The author wishes to express appreciation and indebtedness to Dr. Huntington Williams, Dr. Myron Tull, and Dr. Francis F. Schwentker of the Baltimore City Health Department who provided the opportunity of studying the epidemic.

<sup>2</sup> Type specific antimeningococcus sera with which the precipitin tests were carried out were obtained from the Rockefeller Institute through the courtesy of Doctor Geoffrey Rake.

TABLE II  
Observations on spinal fluid of fatal cases

Case	Age	Precipitin test		Direct smear	Qualitative sugar	Original spinal fluid leukocyte count	Spinal fluid culture	Length of illness prior to admission	Days to sterilize spinal fluid
		10 minutes	1 hour						
H. D...	2 yrs.	+		Many organisms, equally extracellular and intracellular	0	5,300	+	24 hours	Not sterilized 1
M. R...	3 yrs.	++		Moderate number of organisms, mostly intracellular	0	11,000	+	24 hours	
C. J...	6 yrs.	++++		Many organisms, mostly intracellular	0	1,690	+	24 hours	Not sterilized
M. S...	17 yrs.	+++		Loaded with organisms, phagocytosis excellent	0	16,000	+	"several days"	
B. S...	18 yrs.	+++		Many organisms, mostly intracellular	0	40,000	+	48 hours	Not sterilized *
J. C...	18 yrs.	++++		Had appearance of cultural smear	0	800	+	12 hours	
A. G...	18 yrs.	+++		Many organisms, equally extracellular and intracellular	0	16,400	+	8 hours	Not sterilized
C. H...	18 yrs.	+		Many organisms, mostly intracellular	0	36,000	+	48 hours	
L. H...	19 yrs.	++++		Many organisms, mostly intracellular	0	9,600	+	48 hours	Not sterilized
A. S...	22 yrs.	++++		Moderate number of organisms, mostly extracellular	0	400	+	3 days	
E. H...	23 yrs.	+++		Many organisms, mostly intracellular	0	6,700	+	48 hours	Not sterilized 2
G. R...	25 yrs.	+++		Moderate number of organisms, mostly intracellular	0	12,600	+	3 days	
A. H...	30 yrs.	++		Loaded with organisms, mostly intracellular	0	6,200	+	8 days	1
C. B...	37 yrs.	++++		Many organisms, equally extracellular and intracellular	0	6,080	+	24 hours	
J. R...	39 yrs.	+		Very occasional intracellular organisms	Trace	8,800	+	48 hours	Not sterilized
A. B...	42 yrs.	++++		Had appearance of cultural smear	0	1,060	+	24 hours	
S. B...	48 yrs.	++++		Many organisms, mostly intracellular	0	10,400	+	48 hours	Not sterilized 1
Ge. B.	20 mos.	0	0	Very occasional organisms, mostly intracellular	Trace	4,600	+	24 hours	
S. S...	21 yrs.	0	0	No organisms found	+	28	+	24 hours	1

\* Patient died shortly after admission.

meningitis. In general, it appears that the rapidity of appearance of the precipitin reaction is closely correlated with the severity of the infection of the meninges.

In Tables II and III other objective observations made on the spinal fluids are compared with the precipitin results in an effort to assess the prognostic value of each.

The number of organisms present yielded some information of prognostic value. Twenty-one of the 22 cases which gave a positive precipitin test in 10 minutes showed a large number of organisms on stained smears of the spinal fluid sediment. In the group of 47 precipitin negative cases, 16 showed an equally severe infection when this rough method. It is more sig-

nificant that all patients in whom the organisms were rare or absent fell in the negative precipitin group and recovered, with the exceptions of Ge. B. and S. S. where death was the result of obvious invasion of the blood stream. This correlation with the number of organisms present is at best only a rough approximation as neither the time of centrifuging of the spinal fluid nor the amount used was kept constant. It has been found that even when plate counts are made on the spinal fluid the number of organisms varies in different portions withdrawn at one puncture (4). Although the estimation of the number of organisms by this crude method is a matter of routine practice in many clinical laboratories, it obviously fails to provide as accurate a determination of the

severity of the meningeal infection as does the time of appearance of the precipitin test.

The qualitative sugar determination and the cell count on the first spinal fluid were found to be of no prognostic significance. However, the increase in sugar content and the decrease in cell count of subsequent samples were a significant index of response to therapy. With the exception of two patients, A. B. and J. C., where the overwhelming meningeal infection produced a very slight cellular reaction, the degree of phagocytosis was of no value in estimating the severity of infection.

Table IV is the result of an attempt to analyze the duration of infection for evidence of correlation with the intensity of the precipitin test in the 2 groups. It is clear that in the cases studied

there is no indication that the duration of infection plays a significant rôle in the intensity of the precipitin reaction, and it must therefore be concluded either that the more virulent the strain the greater the amount of specific soluble substance produced, or that the quantity depends upon the number of organisms present. This latter must be governed by a host-parasite relationship which is another expression of the virulence. It is clear that primarily the amount of type specific substance in the spinal fluid depends on the number of organisms undergoing lysis. It is, however, possible as some *in vitro* experiments have suggested (5, 6), that a correlation exists between the virulence of the strain and the amount of type specific substance in the antigenic complex. The

TABLE III  
*Observations on spinal fluid of recovered cases*

Case	Age	Precipitin test		Direct smear	Qualitative sugar	Original spinal fluid leukocyte count	Spinal fluid culture	Length of illness prior to admission	Days to sterilize spinal fluid
		10 minutes	1 hour						
E. H...	18 mos.	+++		Loaded with organisms, equally extracellular and intracellular	0	4,700	+	3 days	4
G. M...	19 yrs.	Trace	++	Many organisms, mostly intracellular	0	18,000	+	24 hours	5
J. B...	20 yrs.	+++		Many organisms, mostly intracellular	0	50,000	+	48 hours	4
G. B...	22 yrs.	+++		Moderate number of organisms, mostly intracellular	0	3,200	+	3 days	3
L. F...	37 yrs.	++++		Many organisms, mostly intracellular	0	6,000	+	5 days	6
M. P...	5 mos.	0	Trace	Loaded with organisms, equally extracellular and intracellular	0	5,500	+	3 days	1
E. J...	3 yrs.	0	++	Many organisms, mostly intracellular	0	18,000	+	4 days	1
I. J...	4 yrs.	0	Trace	Loaded with organisms, phagocytosis fair	0	7,600	+	24 hours	1
M. L...	4 yrs.	0	Trace	Loaded with organisms, mostly intracellular	+	4,000	+	24 hours	2
C. M...	9 yrs.	0	+	Occasional organisms, mostly intracellular	0	3,600	+	5 days	1
J. V...	14 yrs.	0	Trace	Moderate number of organisms, mostly intracellular	0	26,600	+	24 hours	1
J. G...	14 yrs.	0	++	Loaded with organisms, mostly intracellular	0	11,000	+	48 hours	1
W. C...	16 yrs.	0	+	Many organisms, equally extracellular and intracellular	0	14,000	+	24 hours	1
L. S...	19 yrs.	0	+	Organisms difficult to find	0	5,760	+	5 days	1
A. M...	26 yrs.	0	+	Moderate number of organisms, mostly intracellular	0	17,200	+	24 hours	1
F. B...	29 yrs.	0	+	Occasional organisms, equally extracellular and intracellular	0	16,800	+	5 days	9
A. O...	29 yrs.	0	+	Many organisms, mostly extracellular	0	7,400	+	4 days	1
A. C...	36 yrs.	0	Trace	Few organisms, mostly intracellular	0	30,100	+	48 hours	1
G. S...	38 yrs.	0	++	No organisms seen	0	5,400	+	4 days	7
F. S...	52 yrs.	0	++	Many organisms, mostly intracellular	0	25,600	+	24 hours	2
B. L...	11 mos.	0	0	Occasional organisms, mostly intracellular	+	5,200	+	24 hours	1
J. S...	20 mos.	0	0	Many organisms, mostly intracellular	0	4,800	+	24 hours	2
Gr. B...	3 yrs.	0	0	Very occasional organisms, mostly intracellular	0	70	+	24 hours	1
R. H...	4 yrs.	0	0	Occasional organisms, equally extracellular and intracellular	0	3,400	+	48 hours	1



TABLE III—Continued

Case	Age	Precipitin test		Direct smear	Qualitative sugar	Original spinal fluid leukocyte count	Spinal fluid culture	Length of illness prior to admission	Days to sterilize spinal fluid
		10 minutes	1 hour						
N. B...	4 yrs.	0	0	Few organisms, equally extracellular and intracellular	+	250	+	24 hours	1
J. J....	6 yrs.	0	0	Few organisms, mostly intracellular	0	5,200	+	6 days	1
W. B...	6 yrs.	0	0	Rare degenerated organisms, all intracellular	0	14,000	0	24 hours	
D. L...	6 yrs.	0	0	No organisms found	+	1,100	+	12 hours	2
E. W...	6 yrs.	0	0	No organisms found	Trace	3,200	0	24 hours	
L. P....	7 yrs.	0	0	No organisms found	Trace	1,000	0	24 hours	
A. D...	9 yrs.	0	0	Occasional organisms, mostly extracellular	0	4,200	+	6 days	1
A. Z....	11 yrs.	0	0	No organisms found	Trace	40,000	0	3 days	
H. P...	11 yrs.	0	0	Occasional organisms, mostly extracellular	+	144	+	8 hours	1
Se. B...	12 yrs.	0	0	No organisms found	0	4,000	+	24 hours	1
P. M...	13 yrs.	0	0	Loaded with organisms, mostly intracellular	0	11,000	+	4 days	4
C. U...	13 yrs.	0	0	Many organisms, mostly intracellular	0	8,800	+	24 hours	1
B. F....	14 yrs.	0	0	Occasional organisms, mostly intracellular	0	12,000	+	24 hours	1
W. L...	15 yrs.	0	0	Moderate number of organisms, equally extracellular and intracellular	0	4,800	+	24 hours	1
A. J....	15 yrs.	0	0	No organisms seen	+	2,400	No real growth* 0	3 days	
A. P...	16 yrs.	0	0	Very occasional degenerated intracellular organisms	+	3,700		5 days	
V. B...	16 yrs.	0	0	Rare organisms, mostly intracellular	0	6,600	+	48 hours	1
An. H...	17 yrs.	0	0	Moderate number of organisms, mostly intracellular	0	2,840	+	2 days	3
B. D...	23 yrs.	0	0	Very occasional degenerated intracellular organisms	0	10,800	No real growth* +	24 hours	
G. C...	23 yrs.	0	0	Rare organisms, mostly intracellular	0	2,800		10 days	1
W. G...	24 yrs.	0	0	Very occasional degenerated intracellular organisms	0	1,400	+	10 days	1
A. K...	26 yrs.	0	0	Rare intracellular organisms	0	5,400	+	48 hours	1
A. N...	30 yrs.	0	0	Many organisms, mostly extracellular	0	6,000	+	5 days	1
F. P....	30 yrs.	0	0	Rare intracellular organisms	Trace	4,500	+	48 hours	1
C. K...	39 yrs.	0	0	No organisms found	0	3,200	0	24 hours	
V. S....	45 yrs.	0	0	Moderate number of organisms, mostly intracellular	0	14,400	+	3 days	1

\* Organisms seen on microscopic smear, failed to grow on second transplant.

TABLE IV

*Duration of infection at time of admission in precipitin positive and precipitin negative groups*

Precipitin test, 10 minutes	24 hours or less	48 hours	3 days	4 days	5 days or more	Total cases
Positive.....	8	7	4	0	3	22
Negative.....	23	7	4	4	9	47

comparison of virulence of strains from the precipitin positive and precipitin negative cases will be reported at a later date.

## SUMMARY

In the study of spinal fluid of 69 cases of meningococcus meningitis prior to treatment, it was

concluded that of the following determinations, spinal fluid cell count, sugar content, degree of phagocytosis, a rough estimation of the number of organisms present, and the time of appearance of the positive precipitin test, the last gave the best indication of the severity of the meningeal infection. When the cases were divided into positive and negative groups on the basis of the result of the precipitin test at the end of 10 minutes, it was found that 77 per cent of the 22 positive cases and 4 per cent of the 47 negative cases were fatal.

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# THERMAL INJURIES: THE EFFECTS OF FREEZING

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The problem of freezing will be considered from two standpoints: first, the local lesion and the therapeutic management of the frozen member, and second, the general effects of freezing including the possible presence of secondary shock and depression of the general body temperature. The literature on freezing as compared with burns is not very extensive. This may be accounted for by the fact that burns are caused by agents of civilization, while freezing usually occurs in out-of-the-way places. It is thus essentially indigenous to localities where medical attention is scant, and except for such rare instances as Beaumont (and Dafoe), empiric rather than physiological observations are made.

## *Local treatment of a frozen member*

One item in the conventional treatment of frozen limbs is the vertical suspension of the limb, especially as practiced by Bergmann (1), König (1) and Selenkoff (1), and discussed by Douglas (3). These authors thought that the viability of the limb is aided by this method. No experiments were performed to test this conclusion. It is possible that one of the factors in frost-gangrene is the compression of the vessels by local swelling, and this could conceivably be aided by vertical suspension. It is interesting to note, however, that vertical suspension is contraindicated in most other forms of impending gangrene as was pointed out by Barney Brooks (4) and others.

A second method in the accepted treatment of freezing is the use of multiple incisions, as applied by Bundschuh (5) and Wittek (6) in 1915. These incisions probably are of value in reducing the local swelling with its resultant pressure on vessels and hence are of value to the viability of the limb. It is to be remembered, however, that the escape of this fluid, while it may be of benefit to the limb, may be a menace to the life of the organism as a whole when severe shock is present.

No experiments were performed to test the efficacy of the incision treatment.

## *Effects of gradual and rapid thawing of frozen tissues*

One of the original purposes of the present work was to test the time-honored conception that a frozen limb must not be thawed out too quickly. No satisfactory physiological support for this empirically adopted custom has been offered. Some observers attribute the danger of sudden thawing to the release of thrombi. Nägelsbach (7) states, however, that he finds neither clinical nor experimental support for this view and that during the World War, the surgeons who adopted the practice of heating frozen limbs as rapidly as possible had at least no poorer results than the others.

In our experiments, both hind limbs of thoroughly anesthetized dogs were frozen stiff by the application of solid carbon dioxide. The legs were not dissected to determine the depth of this freezing, but the joints would not bend and it was believed the legs were frozen entirely through. Then one limb was placed in water at 42° C. for about 20 minutes when thawing was complete and the other in ice water at about 2 to 12° C. for an hour at the end of which time it too had thawed. In the ensuing 18 hours the subsequent behavior of the limbs including reactive hyperemia, restoration of circulation, and secondary swelling was identical.

Both ears of thoroughly anesthetized rabbits were similarly frozen. The amount of freezing was enough so that when thawing was done at room temperature about one-third of the ears would become gangrenous during the next ten days. In a series of eight rabbits one ear was thawed at 38° C. and the other at 2° C. over a period of ten minutes and the ears observed over a period of more than a month. The amount of swelling and subsequent gangrene was essentially similar on the two sides. If anything, the ears

that were thawed in ice water showed a trace more gangrene.

### *Relative effects of freezing by different methods*

Freezing may occur from exposure to cold air, ice and snow, brine ( $-40^{\circ}$  C.), liquid air ( $-140^{\circ}$  C.), solid carbon dioxide ( $-79^{\circ}$  C.) and ethyl chloride. Changes observed clinically following these various types of injury are essentially similar and include freezing of the part followed by reactive hyperemia, edema, bleb formation, and in some cases gangrene. All of the experiments reported in this paper were performed using solid carbon dioxide. The clinical similarity between all types of freezing may be an answer to those who, noting the resemblance between solid carbon dioxide freezing and burns, apply *a posteriori* reasoning and infer that the carbon dioxide produces a burn.

### *Insulating effects of living tissues*

Thermometers were placed in the axillae of 4 dogs, and solid carbon dioxide applied to the surface over the buried thermometer. The average drop in temperature in the first minute was  $11^{\circ}$  C. when the thermometer was placed subcutaneously, and only  $0.2^{\circ}$  C. per minute over the first 10 minute period when the thermometer was placed 2 cm. below the surface. In all instances the skin became solidly frozen before the deep thermometric reading had fallen more than a degree. In one instance at a depth of 2 cm. it took 17 minutes and in another 7 minutes for the local temperature to drop  $10^{\circ}$  C. (a 10 cm. cube of solid carbon dioxide being pressed tightly against the shaved skin). If the measuring thermometer was placed subcutaneously, about 2 minutes' application was necessary to produce a drop in temperature of  $10^{\circ}$  C. in this particular animal. It was found that living tissue was a much better insulator than dead tissue.

### *General changes in body temperature following local freezing*

In animals in which portions of one lateral half of the body were frozen, the rectal temperature was recorded. In animals in which both hind limbs were frozen, the body temperature was de-

termined by inserting a thermometer deep into the muscles through the tracheotomy wound. All of the dogs showed a slight fall in body temperature, but only five fell below  $30^{\circ}$  C. The lowest temperature recorded was  $24.1^{\circ}$  C. ( $75.4^{\circ}$  F.), two hours and forty minutes after beginning the application of ice. Three hours later it had risen to  $35^{\circ}$  C. ( $95^{\circ}$  F.), but fell to  $32.1^{\circ}$  C. ( $89.8^{\circ}$  F.) three hours later at which time the dog was bled to death in order to observe the effect on bleeding volume (see below). Temperatures in the other four animals reached low levels,  $25.7^{\circ}$  C. ( $78^{\circ}$  F.) 16 hours before death,  $25.6^{\circ}$  C. ( $78^{\circ}$  F.) six hours before death by bleeding,  $27^{\circ}$  C. ( $80.6^{\circ}$  F.) three hours before death, and  $24.7^{\circ}$  C. ( $76.5^{\circ}$  F.) one hour before death by bleeding. The course of the temperature readings in four of these animals is shown in Figure 1.

Reincke (8) in 1875 reported a very interesting series from Hamburg, of 17 drunken men exposed to extreme cold while intoxicated. On admission to the hospital all 17 had markedly lowered rectal temperatures. All of the 12 patients with temperatures that did not fall below  $30^{\circ}$  C. ( $86^{\circ}$  F.) recovered. Five patients had temperatures that went below  $30^{\circ}$  C., two of whom recovered. One patient had a temperature of  $28.4^{\circ}$  C. ( $83.1^{\circ}$  F.) 8 hours before death. Another had a temperature of  $30^{\circ}$  C. ( $86^{\circ}$  F.) and recovered. Another case had  $27^{\circ}$  C. ( $80.6^{\circ}$  F.) with death 13 hours later. The most striking case in Reincke's series, however, was that of a 34 year old laborer who was admitted with a temperature of  $24^{\circ}$  C. ( $75.2^{\circ}$  F.). Four hours later the temperature was only  $27.4^{\circ}$ , 10 hours later only  $32.6$ , 23 hours later  $37.8^{\circ}$  C., and on the following day he was discharged completely well. This is the lowest known recorded temperature in a human being with recovery. Sonnenburg and Tschmarke (2) have collected reports of other instances of hypothermia.

Although dogs are perhaps somewhat more poikilothermic than human beings, the temperature of  $24.1^{\circ}$  C. ( $75.4^{\circ}$  F.) in one of our dogs six hours before death by bleeding is of interest. One of the writers (H. N. H.) has seen a case of enormous hydrocephalus (head 75 cm. in circumference) in a child aged 8 months in which the rectal temperature on admission was  $36.7^{\circ}$  C.

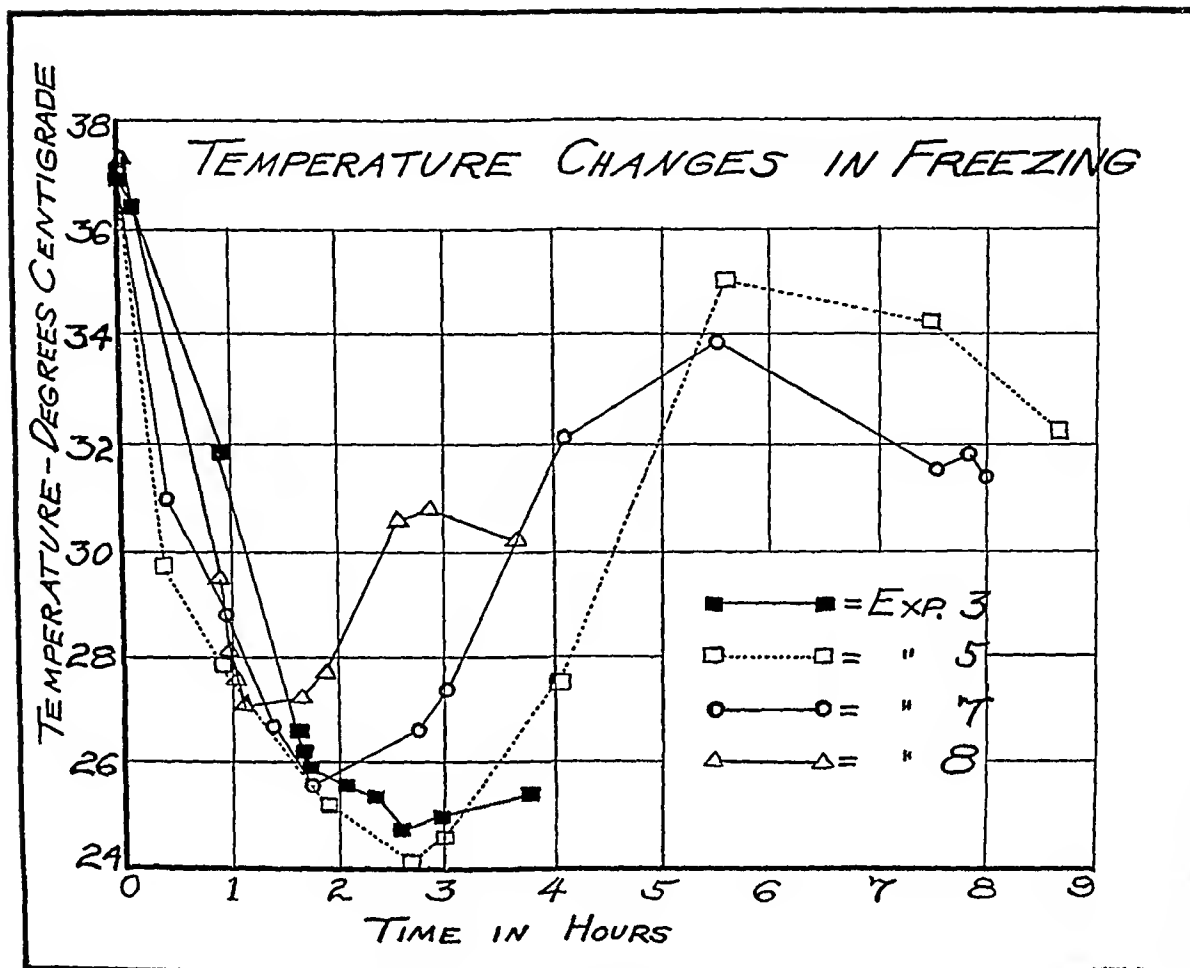


FIG. 1. TEMPERATURE CHANGES IN FREEZING

Dogs 3, 5, and 7 were bled at the termination of the experiment; Dog 8 died. The freezing was induced by application of solid carbon dioxide to the posterior portion of the body, and the temperatures are taken by a thermometer inserted deep into the muscles of the neck and chest. The freezing was usually done over a period of one to two hours.

(98° F.), the next day was 27.5° C. (81° F.) over a four hour period, the third day was 28.0° C. (82° F.) and then rose gradually to 36.6° C. (98° F.) six days after admission when death occurred following spontaneous rupture of the hydrocephalus. Among the lower animals, references are made to transition stages between the poikilothermic condition of the cold-blooded animals and the homothermic state of the higher mammals and birds, in which a constant body temperature is maintained in spite of wide fluctuations in the temperature of the environment. Baldwin (9) has shown that reptiles are not completely cold-blooded. The classic observations of

Kredel (10) on the variations of a representative of the lower mammals, the three-toed sloth, *Bradypus cuculliger cuculliger* Wagler, are of great fundamental importance. This author has carefully recorded the temperature variations of this animal and has shown that the temperature of the air is the chief factor in determining that of the sloth. Some primitive heat regulation comes from within, however.

These experiments are of interest in showing that even in the higher mammals the internal regulation of general body temperature may not entirely control the situation under great stress. This applies equally well to other body functions,

for example the pH of the blood serum. This was formerly thought to remain quite constantly at 7.40 but has been shown to fall to as low as 6.12 under certain temporary conditions (19).

### *Secondary shock in freezing*

It has been shown (11, 12, 13) that in burns there is a marked loss of blood plasma-like fluid from the circulating blood stream into the burned tissues. This plasma loss is associated with a marked blood concentration, fall in blood pressure, lowered bleeding volume and changes suggestive of secondary shock. No reports of shock in freezing were found in the literature, and it was thought advisable to test whether freezing and burning might not produce similar changes. With this in mind the experiments discussed in the following sections were performed.

#### *1. Changes in concentration of blood*

In shock in human beings resulting from severe burns, Underhill et al. (14) found that hemoglobin readings as high as 209 per cent indicated a marked blood concentration. Blalock (11) found that in experimental burns the hemoglobin may rise to 130 per cent. One of the writers (13) found that in experimental burns the hemoglobin may rise to as high as 162 per cent (Sahli: 17 grams per 100 cc. = 100 per cent) and the hematocrit reading to 72 per cent.

Since blood concentration is a regular accompaniment of shock due to burns, it was thought that a similar blood concentration might be present in shock due to freezing. Solid carbon dioxide was applied to about one-fourth of the body surface of 10 completely anesthetized dogs and left in place for about an hour, at the end of which time the underlying tissues were deeply frozen. Frequent blood pressure readings, hemoglobin percentages and hematocrit readings were determined. The blood pressure was obtained by placing in the carotid artery a cannula which was connected to a mercury manometer. Hemoglobin determinations were made by the Sahli method and hematocrit readings with the Van Allen hematocrit.

*Results.* Usually at the end of about 18 to 24 hours the blood pressure fell rather rapidly to an average of about 80 mm. Hg. The changes in

blood concentration began almost immediately after the freezing and were present long before the blood pressure had fallen markedly. It is seen from Table I that in all animals there was an

TABLE I

*Changes in blood concentration in shock due to freezing (the figures recorded after freezing indicate the highest readings in each experiment)*

Experiment	Hemoglobin		Hematocrit	
	Control	After freezing	Control	After freezing
	<i>per cent</i>	<i>per cent</i>		
1	76	131	36	63
2	75	136	37	59
3	102	113	48	58
4	93	104	46	53
5	104	160	48	74
6	97	128	46	63
7	123	159	61	74
8	85	116	42	61
9	96	163	50	70
10	109	168	54	80
Average	96	134	47	66

increase in hemoglobin percentage and hematocrit reading. The average hemoglobin percentage before freezing was 96 and after freezing was 134. The average hematocrit reading before freezing was 47 and after freezing was 66. The figures obtained in Experiment 10 (Table I) are higher than in any readings obtained in shock due to burns, except by Underhill in human beings. Control experiments showed no marked blood concentration (13).

*Conclusion.* There is a marked blood concentration in shock following freezing which is similar in degree to the blood concentration present in shock due to burns. This blood concentration begins before a marked fall in blood pressure occurs and is hence of value in predicting the onset of shock.

#### *2. Shift of body fluids*

It has been shown by Blalock (11), Underhill et al. (12) and the writer (13) that in shock due to severe burns there is a loss of fluid from the blood stream into the burned tissues. It is considered by these authors that this loss of fluids is responsible for a large part of the shock resultant to burns. The amount of fluid shift into the tissues has been measured by burning one lateral

half of an animal and then after careful sagittal bisection, comparing the weight of the burned and unburned sides (11); by weighing the fluid expressed from the water-logged burned tissues (12); and by burning one lateral half of an animal placed on a balanced apparatus and measuring the amount of displacement caused by the increase in weight of the burned side (13).

It was thought that the severe general effects of freezing a portion of the body might be due to a similar leakage of fluid from the blood stream into the frozen or thawing tissues. Portions of one lateral half of 9 completely anesthetized dogs were frozen by the direct application of solid carbon dioxide for about one hour. After a period averaging 21 hours from the time of freezing, 4 of the dogs were dead and the rest were killed. The initial blood pressure in the 9 dogs averaged 147 and the final blood pressure 81 mm. Hg. This amount of blood pressure lowering may be considered as arbitrarily within the limits of so-called surgical shock. The animals were then carefully bisected by the method previously described (13) and a comparison made of the weights of the frozen and unfrozen sides. The average difference amounted to 2.55 per cent of the total body weight. Incision into the tissues that had been frozen revealed sufficient plasma-like fluid to account for the difference in weight. This amount of plasma, if lost from the blood stream, is sufficient to account for a large part of the shock present in these animals (11). In 8 experiments on burns previously reported (13), the amount of fluid shift at death was 2.2 per cent of the total body weight. Control experiments are listed in this previous paper.

*Conclusion.* After freezing, there is a considerable loss of fluid from the blood stream into the frozen tissues. The amount of this fluid shift is similar to that in severe burns and is sufficient to account in part at least for the resultant shock.

### 3. Composition of edema fluid

The composition of the fluid that escapes into the subcutaneous tissues after burns has been determined by Beard and Blalock (15). These authors found that, in general, the chloride content of the fluid was higher than that in blood plasma, the concentration of sugar and nonprotein nitro-

gen was approximately the same in the 2 media and the protein content of the subcutaneous fluid was about 20 per cent lower than that of the blood plasma. Underhill and Fisk (16) made similar comparisons between the tissue fluid after burns and the blood serum. Their results agree in general with those of Beard and Blalock, except that they found the nonprotein nitrogen content considerably higher in the edema fluid than in the blood serum.

TABLE 11

*Shift of body fluids in shock due to freezing*

Experiment	Weight of dog	Duration of experiment*	Blood pressure		Shift of body fluid as percentage of body weight
			Initial	Final	
	kgm.	hours	mm. Hg	mm. Hg	per cent
1	12.2	26	160	114	5.14
2	13.8	28	138	92	0.15
3	16.0	26	164	96	2.44
4	7.3	17	156	94	1.51
5	8.9	20	118	42	4.05
6	11.1	25	168	70	2.92
7	11.0	17	159	38	2.77
8	8.3	13	120	64	1.35
9	7.1	18	142	120	2.59
Average	10.6	21	147	81	2.55

\* The duration of the experiment indicates the time of death of the animal after freezing. The animals in Experiments 1, 2, 3, 4 and 8 were killed immediately following the last blood pressure reading; the animals in the other experiments died sometime following the last reading. The lateral shift of body fluids is determined by the bisection method.

The results of these authors indicated that the fluid that escapes into the subcutaneous tissues after burns very closely resembles blood plasma. The escape of a plasma-like fluid undoubtedly produces more serious consequences than the escape of a simpler solution. Hence, it was considered of importance to determine whether the fluid that escapes into the subcutaneous tissues in large amounts after freezing is of a plasma-like nature similar to that following burns. Nine completely anesthetized dogs in which shock was produced by freezing portions of the body with solid carbon dioxide were dissected after death and large amounts of edematous subcutaneous tissue found. By making multiple incisions in this tissue, sufficient fluid drained out for analysis. This fluid was only slightly tinged with blood, but clotted if no anticoagulant was added. A similar



amount of anticoagulant was added to the edema fluid and to a sample of blood obtained from the carotid artery. Analyses were made of the fluid and of the blood plasma. The sugar was determined by the Folin modification method on a sulfate-tungstate filtrate (20); the sodium chloride by the Eisenman open-Carius method (21); the protein by the Koch-McMeekin micro-Kjeldahl method (22); and the nonprotein nitrogen by the same method on a Folin-Wu filtrate.

TABLE III

*A comparison of the concentration of certain substances in blood plasma and in the fluid that escapes into the subcutaneous tissues after freezing*

Experiment	Sugar		NaCl		Nonprotein nitrogen		Protein	
	Plasma	Fluid	Plasma	Fluid	Plasma	Fluid	Plasma	Fluid
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	grams per 100 cc.	grams per 100 cc.
1		95.2		711.4		38.5		3.9
2	132.5	101.0		665.7	40.0	35.3	4.4	2.9
3		81.0		758.2		36.8		3.2
4	44.1	98.0	673.9	688.0	56.8	50.8	3.3	3.3
5	90.1	13.4	654.0	665.7	95.4	76.3	3.7	3.8
6	84.0	18.9	700.8	703.2	27.0	33.3	4.8	3.6
7	94.2	*	709.0	703.2	45.5	60.6	5.1	3.4
8	177.8	111.1	663.4	690.3	60.3	44.2	3.4	4.7
9		82.1	655.2	686.8		73.2	4.6	3.7
Average	103.8	66.7	676.1	696.9	54.2	49.9	4.2	3.6

\* The value for the sugar in the fluid in Experiment 7 was too low to read.

The results of these analyses are shown in Table III. The concentration of sugar is, in general, lower in the fluid than in the blood plasma. The extremely low values in several instances are of interest. The chloride concentration is approximately the same in the blood plasma and tissue fluid. The nonprotein nitrogen and protein are somewhat higher in the plasma than in the tissue fluid.

Patients with frozen ears were observed frequently, and in all instances after thawing a marked swelling was noted. Only one patient with extensive freezing injury was observed in this study. This patient,<sup>1</sup> a woman, aged 43, tried to commit suicide by lying in the snow a night when the temperature was — 22° F. When

<sup>1</sup> I am indebted to Dr. A. J. Rissinger for permission to report this case.

found, she was not unconscious but both legs were frozen stiff to just below the knees and when her stockings were removed they came off like wall paper from a plastered wall. She received heat generally and cold compresses to the local lesions. The legs thawed out but the toes of both feet and sole of the left foot remained very cyanotic. Later gangrene developed in the toes of the left foot. The blood pressure was normal and there was no blood concentration. One of the chief points of interest with regard to this patient was the extensive swelling and blistering of the frozen legs. This is shown to some extent in Figure 1 which was taken after the largest blister had broken. The blister fluid was somewhat similar to blood plasma on chemical analysis, the total protein being 3.0 grams per 100 cc., the albumin:globulin ratio 2.2, and the nonprotein nitrogen, sugar, and sodium chloride being 26.0, 87.0, and 643.5 mgm. per 100 cc. respectively. One blister contained 40 cc. of this fluid and two other blisters were larger. Measurement of the limbs 36 hours after freezing showed that the volume of the left was greater than the right by approximately 850 cc. Since the right leg was also swollen, this figure may not represent the entire extent of the swelling. Although the plasma loss in this patient was not sufficient to produce shock, it indicates what might happen from freezing of a larger area.

*Conclusion.* The composition of the edema fluid that escapes into the subcutaneous tissues after freezing is quite similar to blood plasma. This indicates that the loss of large amounts of such plasma-like fluid from the blood stream might account, in part at least, for the shock resultant to the freezing. The important difference between the edema fluid produced by freezing and ordinary edema fluid is the high protein content of the former. The rapidity of the loss of this fluid is also of significance.

#### 4. Bleeding volume

The bleeding volume in control dogs was found to be 58.6 per cent of the calculated blood volume (one-thirteenth of the body weight) by Roome, Keith, and Phemister (17) and 53.4 per cent by Harkins (13). On the other hand, the former authors found that in shock due to trauma to an



FIG. 2. BLISTERS AND SWELLING OF LEGS EXISTING 36 HOURS AFTER FREEZING

Both legs were swollen but only the blisters on the left side are well visualized, and the largest of these ruptured before the picture was taken.

extremity, hemorrhage, plasmapheresis, and intestinal manipulation, the bleeding volume was greatly reduced, averaging 21.8 per cent, and in burns the latter author found it to average 20.3 per cent. In another series of experimental burns it was found that in 6 burned animals in which the blood pressure was allowed to fall near a so-called shock level (50 to 82 mm. Hg) before the bleeding volume was determined, the bleeding volume averaged 26.3 per cent. If, however, the bleeding was done before the blood pressure had fallen markedly (102 to 130 mm. Hg), the bleeding volume was already markedly reduced, averaging 31.4 per cent.

The present work was undertaken to determine the bleeding volume in experimental freezing. Portions of the bodies of dogs were frozen by solid carbon dioxide under complete anesthesia (maintained till end of experiment) as described in previous papers (18) and the results shown in Table IV. It is seen that in all instances the bleeding volume was below the normal values.

In three other dogs where the bleeding was

TABLE IV  
*Bleeding volumes of dogs with experimental freezing*

Dog number	Weight	Interval from freezing to bleeding		Blood pressure		Hemo-globin		Hema-tocrit		Terminal bleeding volume
				Start	End	Start	Maximum	Start	Maximum	
	kgm.	hours	minutes	mm. Hg	mm. Hg	per cent	per cent			per cent calculated blood volume
1	7.0	4	45	172	36	86	100	35	50	27.0
2	7.0	15	15	166	72	82	126	39	62	24.2
3	5.7	3	45	148	66	88	100	37	42	39.0
4	7.4	8	10	154	42	99	167	40	72	7.8
5	6.5	9	10	150	82	105	123	43	63	29.9
Average										25.6

done before the blood pressure had fallen markedly (90, 96 and 112 mm. Hg) the bleeding volumes averaged 40.5 per cent (43.1, 31.1 and 47.3 per cent respectively). Two other experiments were of great interest where the blood pressure was rapidly lowered by the general effects of cold before there had been time for considerable fluid exudation. In these two experiments the final blood pressures were 78 and 54 mm. Hg, and the

bleeding volumes determined 2 hours after freezing were 50 and 55 per cent respectively. This indicates that the general effects of cold can reduce the blood pressure before fluid exudation has taken place to any great extent. The fact that at the time of bleeding there were slight local signs of fluid exudation and very little blood concentration also points in this direction. It seems, therefore, that under certain circumstances cold can produce a type of so-called primary shock. If the animal survives this initial period, fluid exudation into the thawing area with blood concentration, lowered bleeding volume and other typical signs of secondary shock ensues.

### 5. Pathological studies

These studies are as yet incomplete. Occasional hemorrhages into the adrenal glands and in one instance a hemorrhage into the duodenal wall, possibly the precursor of a Curling-like ulcer were the chief positive findings remote from the frozen region. At the site of the freezing all blood vessels were found to be patent and no thrombi were found. At the edges of the area the skin was markedly red and injected. In the subcutaneous tissues there was an extensive edema.

### COMMENT

The present work did not help to elucidate the local treatment of a frozen member. Although experiments indicated no difference in the response of dog's legs and rabbit's ears to slow and rapid thawing, the experiments were not conclusive. They did not cover a wide enough variety in extent of freezing and time and rate of thawing, etc. Thus this aspect of the question is still left in abeyance although doubt is cast on the efficacy of the time-honored custom of gradual thawing.

The extreme drop in general body temperature recorded in several of the experiments is of interest. It is quite possible that such low readings may have been influenced by the barbital narcosis, but the restoration of a more normal body temperature in several instances after the removal of the ice augurs against this to some extent. It is quite possible that such low temperatures would not have been obtained if a less severe freezing agent than solid carbon dioxide

had been used. These low readings indicate, however, that even the higher mammals may be relatively poikilothermic under certain conditions.

Aside from the general lowering of body temperature, the presence of secondary shock is of interest in freezing. The lowered bleeding volume, the changes in blood concentration including increase in hemoglobin percentage and hematocrit reading, and the marked leakage of plasma-like fluid into the tissues that have been frozen all indicate that there is a similarity between the effects of freezing and burning. Both of these thermal injuries produce a local reaction and resultant secondary shock. Furthermore, the secondary shock following thermal injury is similar to secondary traumatic shock following other types of injury. The similarity in the reaction of the mammalian organism to injury is most apparent when the comparison is made between the different types of thermal injury. Under natural conditions it is difficult to conceive, however, a situation in which enough of the body could be frozen to produce shock without the effect of exposure to cold being an important if not a dominating factor. In burns, on the other hand, extensive local burning without rise in general body temperature is the rule rather than the exception.

### SUMMARY AND CONCLUSIONS

1. In freezing experiments no evidence was found in favor of the empiric practice of gradual thawing of a frozen member.

2. Under certain conditions freezing and cold cause a marked lowering of general body temperature. Citation is made of Reincke's case of a man with a temperature of 24.0° C. (75.2° F.) who ultimately recovered, and of one animal in the present study with 24.1° C. (75.4° F.) six hours before death by bleeding. *These observations indicate that even the higher mammals may have their temperature regulatory mechanism broken down under a sufficiently severe strain.*

3. Under experimental laboratory conditions a localized portion of the body may be frozen without a predominant general chilling. Under these circumstances a marked exudation of plasma-like fluid into the local tissues occurs with thawing, resulting in blood concentration, lowered bleeding volume and decrease in arterial blood pres-

sure. This condition resembles the secondary shock following burns and indicates the similarity in action of different types of thermal injury. Under very special conditions the same might occur in man.

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# THE ABSORPTION AND EXCRETION OF CALCIUM AND PHOSPHORUS IN THREE PATIENTS WITH COLOSTOMY AND ILEOSTOMY

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It is generally believed that calcium and phosphorus are absorbed from the small intestine and excreted through the kidneys and the colon. The chief factors which influence the absorption from the small intestine are the hydrogen ion concentration of the content of the bowel, the peristaltic rate, and the proportion of the various types of food in the diet. Macfadyen, Nencki and Sieber in 1891 (1), McClendon in 1915 (2) and numerous investigators (3, 4, 5) more recently found the content of the small intestine to be acid most of the time. The strength of the acidity decreases from a pH of about 1.5 in the stomach to approximate neutrality at the ileocecal valve. In spite of this experimental work, one commonly encounters the statement that the content of the small intestine is alkaline. The pancreatic and biliary secretions are in fact alkaline, but these secretions are readily neutralized by excess of acid secretion from the stomach and intestine. The peristaltic rate, or rate of propulsion of the intestinal content, is largely controlled by the nature and bulk of ingested food. A marked increase in the rate of propulsion of content would decrease the time for absorption of calcium and phosphorus as well as other minerals and foodstuffs and would increase the amount of materials retained in the lumen. Diets containing large amounts of fats, excesses of either calcium or phosphorus and highly alkaline diets have been shown by Farquharson, Salter, Tibbets and Aub (6) and others to decrease calcium and phosphorus absorption from the bowel, while acid foodstuffs and proper calcium-phosphorus ratios lead to increased calcium and phosphorus absorption.

In man it has not been possible heretofore to study calcium and phosphorus exchange in the small bowel independent of colonic excretion, due to the difficulty of distinguishing dietary calcium and phosphorus retained in the stool from that excreted into the intestine. An unusual opportunity for study of the interchange of calcium

and phosphorus through the small intestine independent of colonic excretion was afforded by three patients in whom tumor of the cecum necessitated both ileostomy and colostomy, thereby permitting separate collection of colonic and ileal excretion.

## METHODS OF STUDY

After the patients had recovered from operation they were given weighed diets composed of foods of known calcium and phosphorus content. An effort was made to give a diet as near the normal as possible. The urine was collected under toluene in twenty-four hour samples. The excreted ileal content was collected in colostomy pouches which were held against a flat piece of rubber sheeting cut to fit snugly around the base of the colostomy opening by an elastic belt. The flat rubber was pasted to the skin by a layer of yeast between the skin and rubber sheeting. In this manner no material was lost and ulceration of the skin was prevented. The colon was irrigated daily by the injection of 100 grams of distilled water into its proximal opening, and the material was collected as it was expelled through the anus. Usually about 80 per cent of the water was expelled about twenty minutes after its injection. These washings always contained a mucoid, opaque, colorless material free from feces or bile, thus showing the absence of any leakage of ileal content into the colon. The pH of all excreta was determined by the glass-electrode method as modified by Hemingway (7). The ileal content and colon washings were dehydrated either over a water bath or in an electric oven at about 95° C. No attempt was made to dehydrate to a constant weight. The dehydrated material was powdered and one gram samples of the ileal content and all of the colon washings were wet-ashed with concentrated sulphuric and nitric acids. When necessary, superoxyl was added drop by drop to obtain a colorless solution. Aliquot samples of urine were wet-ashed as above. The calcium content of the ashed material was determined by the method of Tisdall and Kramer (8) and the quantity of phosphorus determined by the method of Fiske and Subbarow (9). The ileal content corresponding with any experimental period was marked with carmine or charcoal. The identifiable substances appeared at the ileal opening two to three hours after oral ingestion. Controls on results of analyses consisted of reagent blanks, known solutions, and known solutions added to unknowns run simultaneously with the unknowns. Results not checking to within five per cent were repeated except in the colon washings,

where larger variations were expected because of the very low calcium and phosphorus content of the material analyzed.

*Case 1.* G. M., a 62 year old white male who was in good health until 1930, when he experienced heart burn and gas pains following meals. January, 1934, he noticed a progressive weakness and weight loss which by March, 1934, compelled him to stop work. Examination March, 1934, revealed weight loss and a palpable tumor mass in the right lower quadrant. There was an associated melena and a secondary anemia, hemoglobin 46 per cent, (Sahli, 17 grams per 100 cc. blood equals 100 per cent) and 4.75 million erythrocytes per cubic millimeter of blood. X-ray examination of the colon revealed a filling defect in the cecum through which peristalsis did not pass. March 28, 1934, the cecum, the proximal four inches of the ascending colon, and the terminal six inches of the ileum were resected because of an adenocarcinoma (microscopic diagnosis) of the cecum. The free ends of the colon and ileum were brought to the surface through a colostomy opening. One month later, when the anemia was corrected and peristalsis was normal, the patient was used for these studies. This patient is well (November 1936), and no evidence of recurrence or metastasis has been found.

*Case 2.* Mrs. A. R., age 63, had been constipated for twenty-five years. Prior to the last few years the patient had an occasional attack of pain in the right upper quadrant associated with chills, fever, and less often, jaundice. In 1932 she began having attacks of intermittent colicky pain in the right lower quadrant of the abdomen associated with nausea, vomiting, and abdominal distention. These attacks came on more often during 1934. She had lost twenty-two pounds of weight during the four months prior to admission in November, 1934. There was a firm, nodular, fixed mass in the right iliac fossa. The size of this mass varied considerably from time to time. X-ray examination of the colon revealed a constant irregular filling defect through which peristalsis did not pass. No shadow of the gallbladder was found at any time during a cholecystographic study. The hemoglobin was 66 per cent (Sahli), and the red blood cell count was 3.3 million per cubic millimeter of blood. Other laboratory tests were normal. December 1934, six inches of terminal ileum, the cecum, and about six inches of ascending colon were resected because of a suspected carcinoma. The mass on microscopic examination proved to be a carcinoid tumor of the cecum. The gallbladder at operation was thickened and contained stones but was not removed. This patient's convalescence was greatly prolonged because of inability to retain adequate amounts of food. She also lost large amounts of fluid through the ileostomy opening until it was closed. She was in good health in November, 1936.

*Case 3.* Mrs. M. M., age 50, noticed a small mass in the right lower quadrant in the fall of 1934 which produced very few symptoms. In February 1935, she experienced alternating periods of diarrhea and consti-

pation. The mass began to grow rapidly. Examination at that time showed a somewhat undernourished pale female with movable mass in the right iliac fossa, about three inches in diameter. X-ray examination revealed a filling defect in the cecum which could not be differentiated from a granuloma, carcinoma, or carcinoid of the cecum. Hemoglobin was 57 per cent (Sahli) and there were 2.62 million red blood cells per cubic millimeter of blood. There was occult blood in the feces; other tests and examinations gave normal or negative results. At operation, a large tumor mass was found involving the cecum and appendix. The cecum and the adjacent ten inches of terminal ileum and nearly all of the ascending colon were resected. The proximal end of the colon and the terminal end of the ileum were brought to the surface through a colostomy opening. On microscopic examination the tumor proved to be a gelatinous carcinoma of the cecum. The immediate convalescence was uneventful, but about one year later the patient developed bone metastasis for which she was given roentgen-ray therapy. The patient died from cachexia on June 13, 1936. Postmortem examination confirmed the above diagnosis.

## RESULTS

The patient G. M. was able to follow prescribed diets, but became very restless and refused to remain sufficiently long to permit more than one three-day period of study without change of procedure. In Patient A. R. a calcium and phosphorus balance study was made before the operation with considerable difficulty because constant persuasion was necessary to get the needed cooperation. Following operation the patient refused to eat any constant diet; in fact, her stay in the hospital was prolonged for months because of anorexia and a marked diarrhea through the ileal opening with a loss of large amounts of ileal content until the ileum and colon were anastomosed. However, colonic washings were satisfactorily obtained. Samples of ileal content were obtained from the ileal opening at various times for determination of the pH, but a complete collection was not possible. Satisfactory colon washings were obtained on five occasions. Table I lists the volume and pH of urine obtained from Patient G. M. and the daily amount of ileal elimination in terms of wet and dry weights. The colon washings were similarly measured and recorded. The pH was determined on freshly obtained wet materials from the ileum and colon. The patient G. M. was given a diet containing food and water as indicated in Table I. The diet

was changed two days before the beginning of the first and third periods, thereby reducing the error introduced by excreta from a previous period being mixed with that of a subsequent period. The urine volume follows closely the fluid

intake during the first three periods. The urine collection of 215 cubic centimeters during April 30 was incomplete since the creatinine, calcium, and phosphorus excretion in that specimen was too low when compared with other collections of

TABLE I

*The quantity and pH of urine, ileal content, and colon washings obtained from patients with colostomy and ileostomy openings*

Date	Daily intake		Urine		Ileal elimination			Ratio of wet weight to dry weight	Colon washings returned		
	Water	Weight of food as served	Cc.	pH	Wet	pH	Dry		Wet	pH	Dry
		grams			grams per 24 hours		grams per 24 hours		grams		grams
CASE G. M.											
April 29, 1934	750 cc. Control period	609	246		273.7		23.1		70.0		1.27
April 30, 1934			215*		298.0		24.1		97.0		1.52
May 1, 1934			270		292.4		23.2		72.5		1.49
Average			263		288.1		23.5	13.3			
May 2, 1934	2000 cc.	609	1200		316.4		27.5		94.5		1.27
May 3, 1934			1808		295.5		21.9		89.2		1.25
May 4, 1934			1555	5.4	354.8	5.4	26.2		50.7	8.3	0.64
Average			1521		322.2		25.2	12.8			
May 7, 1934	1500 cc.	812	331	5.6	410.5	5.3	31.8		62.2	8.2	1.00
May 8, 1934			713	6.4	418.0	5.7	28.3		95.3	8.3	1.49
May 9, 1934			837	6.8	442.4	5.6	32.3		97.0	8.0	1.37
Average			627		423.6		30.8	13.8			
May 10, 1934	1500 cc. Viosterol 750 D (3 cc.) t.i.d.	812	938	6.9	346.3	5.4	25.7		49.1	8.2	1.15
May 11, 1934			1105	6.6	372.5	5.6	31.1		103.5	8.0	1.50
May 12, 1934			1553	6.4	392.1	5.5	29.6		97.0	7.2	1.47
Average			1199		370.3		28.8	13.0			
May 13, 1934	1500 cc. Parathormone 40 units b.i.d.	812	1533	6.5	241.0	5.0	26.1		89.7	8.0	1.22
May 14, 1934			2140	6.4	231.4	5.3	25.8		41.6		0.98
May 15, 1934			1043	6.5	215.0		24.5		84.2	8.2	1.08
Average			1572		229.1		25.5	9.0			
CASE A. R.											
February 5, 1935 to February 11, 1935	Inconstant diet				Very large volume 2000 to 4000 grams 1369 to 1575 grams	6.07 to 6.68			150 50	8.6 8.3	
April 6, 1935 to April 11, 1935											
						6.37 to 6.84			60 76 58	8.2	
CASE M. M.											
March 14, 1935 March 15, 1935 March 17, 1935	Not controlled					7.63 6.56 7.09			44 6.7	8.7 8.3	

\* Urine collection incomplete—Values not used in obtaining average values.



that period. No adequate explanation is apparent for the small twenty-four hour urine collection of 331 cc. on May 7, 1936. It was complete, for the creatinine and mineral excretion values were comparable with those of other collections for the same period. The fourth and fifth periods show diuretic effects of viosterol and parathormone. pH determinations were not made during the early part of this study because the glass electrode potentiometer was not available.

The amount of ileal elimination varied directly with the quantity of water and food ingested. Food and fluid when taken in the usual amounts tended to increase peristalsis and decrease the time for absorption from the small intestine. This point is well illustrated by comparing the first period with the second. The diet was identical in both periods excepting that during the second period water consumed was increased from 750 cc. to 2000 cc. daily. This resulted in an increase in average daily ileal excretion of 34.1 grams wet weight and only 1.7 grams of dry substance. The ratio of dry to wet ileal excretion increased from 1 to 13.3 to 1 to 12.8, showing that the increased elimination was chiefly water loss. During the third and subsequent periods the bulk of the diet was increased by one-third, four meals were given instead of three. With this increase in bulk there was an increase in both wet and dry substance in the ileal excretion. The ratio of dry substance to wet weight was 1 to 13.8. Since both the bulk and water content of the diet were changed in this period, a simple comparison with the previous two periods is not possible. When viosterol was added to this diet (next period), a diuresis resulted, and there was a marked decrease in the wet weight and smaller decrease in the dry weight of the ileal excretion, the ratio changing from 1 to 13.8 to 1 to 13. The ileal excreta was less fluid than previously, but still was liquid. During the last period viosterol was discontinued and parathormone was injected. The patient became dehydrated as indicated by a dry skin, a cessation of gain in weight, a large urinary output, and sensation of thirst. The colon washings at this time contained formed plugs of mucus which previously had been liquid. The ileal excreta was semi-solid and almost pasty. The wet weight of the ileal excreta decreased from 370.3 grams to

229.1 grams with a less marked decrease in the elimination of dry substance (from 28.8 grams to 25.5 grams). The dry to wet ratio increased from 1 to 13 to 1 to 9. The absolute decrease in dry substance indicates a better absorption from the small intestine. These effects from viosterol and parathormone administration probably represent a more rapid absorption of water due to hemoconcentration resulting in a slower rate of propulsion of ileal content and a longer time for absorption of foodstuffs from the small intestine rather than any specific effect of viosterol or parathormone upon the mucosa of the intestine. Later, it will be shown that the decrease in both calcium and phosphorus content of the ileal excreta after viosterol and parathormone administration parallels decreased water content.

The content of the terminal ileum in the case of G. M. was quite acid at all times. As previously stated, the daily ileal elimination was collected in two portions of approximately twelve hours each, the 7 a.m. to 7 p.m. collection representing chiefly the food residue and the 7 p.m. to 7 a.m. representing the fasting excretion. The pH of the excreta collected during the period of fasting averaged about 0.2 lower (more acid) than the dry excreta. This difference became more marked when samples taken three to four hours after meals, a time when maximum elimination occurred, were compared with pH readings from material obtained after fourteen hours of fasting. Following meals, the material became about neutral, readings of 6.5 to 6.9 were obtained and readings as low as 4.3 were obtained during the fasting state. In the case of A. R. the daily ileal elimination was 1300 to 1600 grams. The patient was unable to take a constant amount of either fluid or food preventing any balance study. The pH of the content of the terminal ileum varied from 6.07 to 6.8, a slightly acid reaction. These readings for the most part represent fasting values, since the patient was able to take but little food until after the ileostomy was closed. The last patient, M. M., found it necessary to take a very high bulk coarse diet to prevent a large fluid loss from the ileostomy opening. It is possible that this diet may have been a factor in producing an approximately neutral reaction of the ileal content. This is

suggested by the above mentioned observation of a tendency for the content to approach neutrality following meals and becoming decidedly more acid in the fasting state. It will be noted in the case of G. M. that an increase in dietary bulk did not influence the average pH; however, the ratio of bulk to other foods remained the same. The

difference in reactions of the ileal content in these cases may represent individual differences. Robinson (10), working with dogs, concluded that the pH of the content of any segment of small intestine was controlled by secretions from the wall of that segment. Of course, any rapid ingress of intestinal content having a decidedly different

TABLE II  
*The calcium and phosphorus content of the excreta*

Date	Daily intake		Urine		Ileal elimination		Colon washings		Total excretion		Balance	
	Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus
	grams per day	grams per day	grams per day	grams per day	grams per day	grams per day	grams per day	grams per day	grams per day	grams per day	grams per day	grams per day
CASE G. M.												
April 28, 1934	.55	.74	.17	.64	.42	.18	.016	.003				
April 29, 1934	H <sub>2</sub> O 750 cc.		.16	.51	.40	.17	.022	.003				
April 30, 1934			.14*	.38*	.38	.21	.007					
May 1, 1934			.18	.61	.40	.20	.003	.002	.57		-.02	
Average			.17	.59	.40	.20				.79		-.05
May 2, 1934	.55	.74	.20	.80	.44	.20	.003	.002				
May 3, 1934	H <sub>2</sub> O 2000 cc.		.16	.62	.41	.20	.003	.003				
May 4, 1934			.18	.67	.56	.22	.002	.004	.65		-.10	
Average			.18	.72	.47	.21				.93		-.19
May 7, 1934	.73	1.00	.13	.62	.60	.24	.004	.005				
May 8, 1934			.13	.64	.55	.25	.004	.004				
May 9, 1934			.14	.65	.62	.26	.004	.003	.72		+.01	
Average			.13	.64	.59	.25				.89		+.11
May 10, 1934	.73	1.00	.15	.65	.55	.23	.005	.004				
May 11, 1934	Viosterol 750 D		.17	.68	.60	.27	.004	.004				
May 12, 1934	(3 cc.) t.i.d.		.18	.86	.61	.27	.003	.003	.76		-.03	
Average			.17	.73	.59	.26				.99		+.01
May 13, 1934	.73	1.00	.20	1.36	.53	.24	.003	.003				
May 14, 1934	Parathormone		.35	1.43	.55	.29	.005	.003				
May 15, 1934	40 units b.i.d.		.47	1.06	.43	.20	.004	.004	.84		-.11	
Average			.34	1.28	.50	.24				1.52		-.52
CASE A. R.												
February 12, 1935							.017	.051				
February 14, 1935							.063	.009				
April 6, 1935							.008	.005				
April 7, 1935							.005	.004				
April 10, 1935							.001	.001				
CASE M. M.												
March 16, 1935							.001	Trace				
March 17, 1935							.002	.003				

\* Urine collection incomplete—Values not used in obtaining average values.

reaction into another segment of bowel would greatly modify the pH of the content of the receiving segment.

The character, gross appearance, and pH of the colon washings were very similar in all three cases and consisted of white mucin-like material which became less fluid when the patient was dehydrated. Staining and contamination by ileal content was not observed at any time. The pH of material obtained from all three patients varied but little (8.0 to 8.7).

Calcium and phosphorus absorption and excretion data from analyses of urine, ileal and colonic excreta are listed in Table II. Patient G. M. was used for a calcium and phosphorus balance study. As previously stated, the other patients were not suitable for a similar study. The daily values are listed and averaged for each period. This was thought advisable since average figures do not give any information regarding daily variation within a period. Had it been possible to run more than one three-day period on each variation of the study, the listing of daily values would not have been advisable. During the first period and for the preceding two days the patient was given a diet containing 0.55 gram calcium and 0.74 gram phosphorus per day; the average daily urinary excretion of calcium was 0.17 gram and 0.59 gram of phosphorus. The daily ileal excreta contained 0.4 gram calcium and 0.2 gram phosphorus, and the material obtained by washing out the colon contained only a few milligrams of calcium and phosphorus. It is noteworthy that the colon washings did not contain any significant amount of calcium or phosphorus at any time even though during the last period the serum calcium was elevated to 15.3 mgm. per cent by parathormone injection, which resulted in a very significant increase in urinary calcium and phosphorus excretion. The intake of calcium and phosphorus during the second period was the same as during the first, the only change was an increase of water from 750 cc. to 2000 cc. daily. The slight increase of urinary calcium excretion is probably of no significance, but the increase of urinary phosphorus excretion from 0.59 to 0.72 gram per day is a significant one and corresponds to the increase commonly encountered when diuresis occurs. The increase in daily ileal calcium

elimination parallels reasonably well the increase in dry material excreted as shown in Table I. The increase in urinary phosphorus and ileal calcium elimination during this period resulted in a small negative balance. It was necessary to increase the diet to permit a gain in weight and to satisfy the patient. This was accomplished by increasing the number of daily meals to four without otherwise changing the diet. Two days after this change the third period was begun. During this period the patient was in a state of calcium balance and a positive phosphorus balance of 0.1 gram per day. When viosterol was added during the fourth period, the urinary excretion of calcium and phosphorus increased while the ileal excretion remained constant, converting the calcium balance to a slight loss and the previously positive phosphorus balance to a balanced state. During the last period the parathormone injections, as previously stated, resulted in a hypercalcemia and a moderately severe dehydration of the patient. There resulted a marked increase of daily urinary calcium and phosphorus excretion and a decrease in ileal calcium and phosphorus elimination which produced a negative balance of both calcium and phosphorus. In Patients A. R. and M. M., except for the first washings in the case of A. R., the colon washings did not contain any appreciable quantities of calcium and phosphorus. The amounts of calcium and phosphorus excreted in the colon washings in all three cases is surprisingly small.

#### DISCUSSION

Vitamin D is commonly believed to increase absorption of calcium from the small intestine. As shown in Table II when viosterol was administered, there was a significant decrease in both the dry and wet weight of the ileal excreta. It was suggested that this possibly was due to a slowed rate of propulsion permitting a longer time for absorption of absorbable material from the small intestine. It was also pointed out that taking of larger amounts of water as in the second period of Table I resulted in a larger wet and dry ileal elimination and that clinical signs of dehydration and a marked decrease in both the wet and dry ileal excretion resulted when parathormone was administered. If these variations were a result

of greater or lesser absorption from the small intestine dependent upon rate of propulsion and time for absorption rather than selective action of viosterol or parathormone upon the mucosa of the intestine, then the mineral excretion should have paralleled the dry weight elimination. Table III lists these data. On the first diet there

TABLE III

*Milligrams calcium and phosphorus contained in each gram of dry weight of average daily excreta*

	Dry weight of excreta	Daily ileal excretion		Dry weight	
		Calcium	Phosphorus	Calcium	Phosphorus
	grams	mgm.	mgm.	mgm. per gram	mgm. per gram
Control period.....	23.5	400	200	17	8.5
Period of hydration.....	25.2	470	210	19	8.3
Diet changed					
Control period.....	30.8	590	250	19	8.1
Viosterol (3 cc.) t.i.d. 750 D.....	28.8	590	260	20	9.0
Parathormone 40 units b.i.d.....	25.5	500	240	20	9.4

were excreted in each gram of dry material 17 and 8.5 mgm. of calcium and phosphorus respectively. When the water intake was increased and the diet otherwise remained constant, there was a greater increase of ileal calcium excretion or less absorption of calcium than during the control period. The phosphorus excretion was not appreciably altered. With the patient on four meals daily and on intermediate water intake, the excreta contained 19 and 8.1 mgm. calcium and phosphorus respectively. Viosterol was then added to the diet, and even though there was a decrease in the grams of dry excreta, the concentration of calcium and phosphorus in this excreta increased. A similar but even more marked effect was noted when viosterol was discontinued and parathormone administered. These data do not support the contention that viosterol increases the absorption of calcium specifically from the intestine, but indicate that through a general increase of absorption from the small intestine there may result some increase in mineral absorption including calcium. It is possible that this increase in absorption is due to slowed rate of

propulsion and an increased time for absorption from the intestine.

### CONCLUSIONS

In two of three patients studied, the content of the terminal ileum was acid at all times, and in the third patient the content was usually acid, but following the rapid expulsion of very coarse vegetable fiber, the content became approximately neutral.

Following the ingestion of a large amount of water, the ileal excreta became more fluid, and there was an increase in weight of the wet, dry and mineral excretion.

Viosterol and parathormone administration resulted in a diuresis and a concomitant decrease in the wet and dry weight of ileal excretion. The ileal content became thick and semisolid whereas it had been quite fluid on the same diet during the preceding period. The more concentrated ileal excreta contained more calcium and phosphorus per gram of dry weight than the hydrated.

No evidence was obtained that viosterol in the amounts given to this patient had any specific effect on the absorption of calcium from the intestine.

In these three patients the colon failed to excrete significant amounts of calcium and phosphorus at any time during the study.

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# THE GLYCINE SYNTHESIS IN PATIENTS WITH PROGRESSIVE MUSCULAR DYSTROPHY

By AXEL THOMSEN

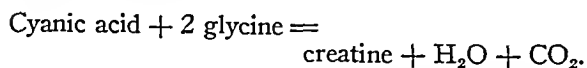
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In 1929 Brand, Harris, Sandberg and Ringer (3) demonstrated that in patients with progressive muscular dystrophy, they could raise the excretion of creatine in the urine, characteristic of these patients, to 40 per cent above the normal by feeding glycine, while other amino-acids were without effect in this respect.

On repeating these experiments three years later, Thomas, Millhorat and Techner (9) found that the patients definitely improved when the administration of glycine was continued for some length of time, and they suggested that this beneficial effect might be explained by assuming that a reduced ability to synthesize glycine was an essential point in the pathogenesis of the disease. This would imply that glycine, introduced from without, would act as a substitute for the organisms own insufficient production. If glycine be accepted as a precursor of creatine, the theory would explain the raised creatine excretion following glycine administration to these patients.

In their first paper, Brand and coworkers (3) advocated the theory, based upon the Salkowsky-Werner-Fosse cyanic acid theory, that creatine might arise from a side reaction between cyanic acid and glycine:



In more recent papers by Brand (1) and by Brand and Harris (2) the theory is somewhat altered. It is still assumed that glycine is involved in the formation of creatine, namely, of its guanidine group, but for special reasons the glutathione, which is known to contain glycine, is put in relation to the process.

Since publication of the study by Thomas and his coworkers (9) many patients suffering from progressive muscular dystrophy have been treated with glycine, and although there is some division of opinion as to the therapeutic effect, most authors confirm Brand's observation as to the raised

creatine content in the urine following glycine feeding.

A theory based on the assumption of a defective glycine synthesis is in itself rather surprising, since it is a generally accepted view that glycine, at least, is an amino-acid which the organism itself can synthesize. But naturally the idea could not be dismissed at the outset, that certain individuals did not possess this function, and this deficiency might be the cause of a disorder as grave as progressive muscular dystrophy.

If benzoic acid be given to a person, the main part combines with glycine and is excreted as hippuric acid. This function seems to extend to many members of the animal kingdom (pig, rabbit, guinea pig, man), and to be a very adequate function, allowing great amounts of benzoic acid to be eliminated. McCollum and Hoagland (7) succeeded in showing, with pigs reduced to an endogenous level of protein metabolism, that the formation of hippuric acid could proceed at a constant level of nitrogen excretion, but was accompanied by a great reduction in the excretion of urea. Only when the urea-nitrogen was lowered to 20 per cent of the total nitrogen was the nitrogen excretion increased, indicating an increased breakdown of protein.

In their first studies Brand, Harris, Sandberg and Ringer (3) fed benzoic acid to their patients with progressive muscular dystrophy. The hippuric acid excretion is not mentioned, but they conclude: "The transformation of glycine into creatine was further confirmed by the drop in creatine excretion following benzoic acid and sodium benzoate feeding." In 1933 Harris and Brand (5) write: "It was repeatedly found that the administration of benzoic acid produced a prompt and appreciable decrease in the creatinuria," and this statement is illustrated by a chart. In 1933 Shorr, Richardson and Wolff (8) find, in patients with Graves' disease, a sharp increase in creatine excretion following sodium benzoate

feeding. With the exception of the above mentioned chart by Harris and Brand (5) none of these authors publishes data in support of their assertions.

In 1933 Freiberg and West (4) published their investigations on the formation of hippuric acid in 3 children with muscular dystrophy, using 3 normal children of corresponding age and weight as controls. The amounts of benzoic acid fed, 0.1 gram per kgm. of body weight, are rather insignificant, but their results show that the patients synthesize hippuric acid, and hence glycine, at the same rate as the normal controls. Their results are collected in Table I, supplemented by my calculations of the hippuric acid that could have been produced from all the benzoic acid ingested, and the amount of glycine present in the hippuric acid excreted.

TABLE I

*Data from Freiberg and West (4). Glycine synthesis in 3 children with progressive muscular dystrophy after benzoic acid feeding*

	Age	Body weight	Benzoic acid ingested	Hippuric acid		Glycine	
				Found	Calculated	Found	Calculated
	<i>years</i>	<i>kgm.</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
Patient 1	11	34.6	3.46	4.698	5.52	1.81	2.13
Control 1	12	36.0	3.60	4.108	5.75	1.58	2.21
Patient 2	9	28.6	2.86	3.214	4.56	1.24	1.76
Control 2	8	27.2	2.72	3.050	4.35	1.18	1.67
Patient 3	8	21.6	2.16	2.436	3.45	0.94	1.33
Control 3	8	22.5	2.25	2.847	3.60	1.08	1.38

As will be seen from Table I, the authors (4) cannot account for all the benzoic acid ingested, but this may be caused partly by the fact, which their curves seem to show, that the excretion did not stop altogether within the time of sampling (5 hours). A more serious objection is that these small amounts of benzoic acid cause only very minute amounts of glycine to be produced. If the therapeutic dose for children of this age is about 15 grams per day, the experiment must show that the diseased organism can synthesize amounts of about the same order.

It may likewise be objected that the glycine present in the hippuric acid might have been extracted from the patients own proteins, which contain on an average about four per cent of

glycine. To guard against such an objection, it is necessary to determine the nitrogen excretion simultaneously. The organism being without glycine-deposits, such an extraction of glycine from the protein molecules must of necessity mean the total disintegration of the molecules, with consequent increase of nitrogen excretion to a level twenty-five times as great as the nitrogen content of the glycine extracted.

In 1934 Linneweh and Linneweh (6) published their results from feeding benzoic acid to two adult patients with progressive muscular dystrophy. They determined the nitrogen excretion simultaneously with the hippuric acid excretion, and found that the hippuric acid excretion was paralleled by a rise of nitrogen excretion in the urine, corresponding fairly well with the nitrogen content of the hippuric acid. While it must be admitted that their doses of benzoic acid are fairly large, it can only be regretted that in one of their patients the hippuric acid output is rather poor, 22 grams only, while 48 grams should have been expected from the 30 grams of benzoic acid ingested. It might therefore equally well be concluded that the hippuric acid synthesis is impaired in this patient. Their data have been arranged in Table II, together with the supplementary calculations.

TABLE II

*Data from Linneweh and Linneweh (6). Glycine synthesis in 2 adult patients with progressive muscular dystrophy after benzoic acid feeding*

	Benzoic acid ingested	Hippuric acid		Glycine	
		Found	Calculated	Found	Calculated
	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
Control.....	30 (?)	36	48.0	13.85	18.50
Patient 1.....	16	25	25.6	9.60	9.85
Patient 1.....	30	41	48.0	15.80	18.50
Patient 2.....	16	18	25.6	6.92	9.85
Patient 2.....	30	22	48.0	8.46	18.50

These authors also determined the creatine and creatinine excretion during the experiments, and they conclude that the creatinuria is not reduced after feeding benzoic acid.

As will be seen from these quotations, the literature is contradictory on one point, viz., the question of reduced creatinuria following the

feeding of benzoic acid, while on another, the synthesis of hippuric acid, no indisputable conclusion can be drawn. It has been the object of the experiments now to be described, to throw further light on these problems.

### EXPERIMENTAL

The experiments were performed on six patients with typical cases of progressive muscular dystrophy, who were admitted to the Department of Nervous Diseases and the Medical Department A of the Rigshospital (Experiment II).<sup>1</sup> On the day of the experiment and the day preceding, the patients were served a glycine-free diet consisting of milk, cream, butter, eggs, sugar, bread baked from potato meal and whites of egg, and courses prepared from these ingredients with the addition of salt and spices. No attempt was made to make the nitrogen intake constant, because of practical difficulties; and because, after all, the nitrogen excretion very soon proved to be fairly constant.

It was my aim to give the greatest possible amount of benzoic acid during the day of the experiment, in order to get an acceptable amount of glycine synthesized. It was therefore indispensable to determine (Experiment I), the largest dose of benzoic acid (given as sodium benzoate) which could be taken without substantial discomfort, together with the duration of its excretion.

### METHOD

None of the methods described for the determination of hippuric acid seem to be generally accepted, nor do they give very accurate results, as has been shown in the preceding part. I have preferred the method described by Widmark (10), the principle of which is titration of the benzoic acid after extraction with toluol, in which the hippuric acid is almost insoluble. Instead of the large extraction vessels described by Widmark, I used the smaller tubes constructed by Ørskov (11) for similar purposes. They possess great practical advantages. From the microburette 0.1884 cc. of 0.05 N NaOH is measured into one end of the extraction tube (the recipient), while into the other (the dimittent) is measured 0.8 cc. of the urine, which has previously been acidified with sulphuric acid to about 0.5 N. The tube is filled up

with toluol and rocked for four hours. After this extraction, the toluol plus the content of the dimittent is sucked off and a surplus of 0.05 N HCl is added to the recipient from a microburette. It is heated on a water bath at 50 to 60° C., and a current of air is blown through the recipient to expel the carbon dioxide. The acid is then titrated with 0.05 N NaOH from the microburette, using phenolphthalein as an indicator.

Each sample of urine was extracted twice, the first time for the determination of "preformed benzoic acid" directly on the acidified urine, the second time for the determination of the "total benzoic acid" after hydrolysis of the hippuric acid.

The heading "preformed benzoic acid" is a purely practical one, as it covers widely different substances, viz., 1, free benzoic acid; 2, ester of benzoic acid and glucuronic acid, which disintegrates immediately when sulphuric acid is added; 3, hippuric acid which, as already mentioned, is not entirely insoluble in toluol; 4, other acid substances soluble in toluol.

Although the fraction is small in comparison with the hippuric acid fraction, it would seem desirable, from a theoretical standpoint, to determine the single components. The greatest is, no doubt, the hippuric acid, since preliminary experiments had shown it to be so soluble in toluol that a not inconsiderable part would be dissolved, when the urine was rich in it. Next, an attempt was made to determine the ester fraction by employing a titration with Benedict's solution. But it appeared that the "Eigenreduction" of the urine was so considerable in comparison with the small amount of glucuronic acid present that the plan had to be abandoned. The free benzoic acid eludes direct determination, but it is probably rather insignificant in amount. This can be said with certainty about the "other acid substances," since this component cannot be expected to increase *after* benzoate feeding, and *before* such feeding the whole fraction of "preformed benzoic acid" is almost negligible. One must, therefore, be content to determine the sum of the components, and to compromise with the inaccuracy introduced by assuming that all the benzoic acid determined as "total benzoic acid" originates from hippuric acid.

"The total benzoic acid" is determined after treating equal amounts of urine with 4 N NaOH on a boiling water bath for six hours. The fluid is neutralized with 2 N H<sub>2</sub>SO<sub>4</sub>, and after acidify-

<sup>1</sup>I am indebted to the chiefs of the two departments, Professor Dr. med. Viggo Christiansen, and Professor Dr. med. Carl Sonne, for their kind permission to perform these experiments on their patients.



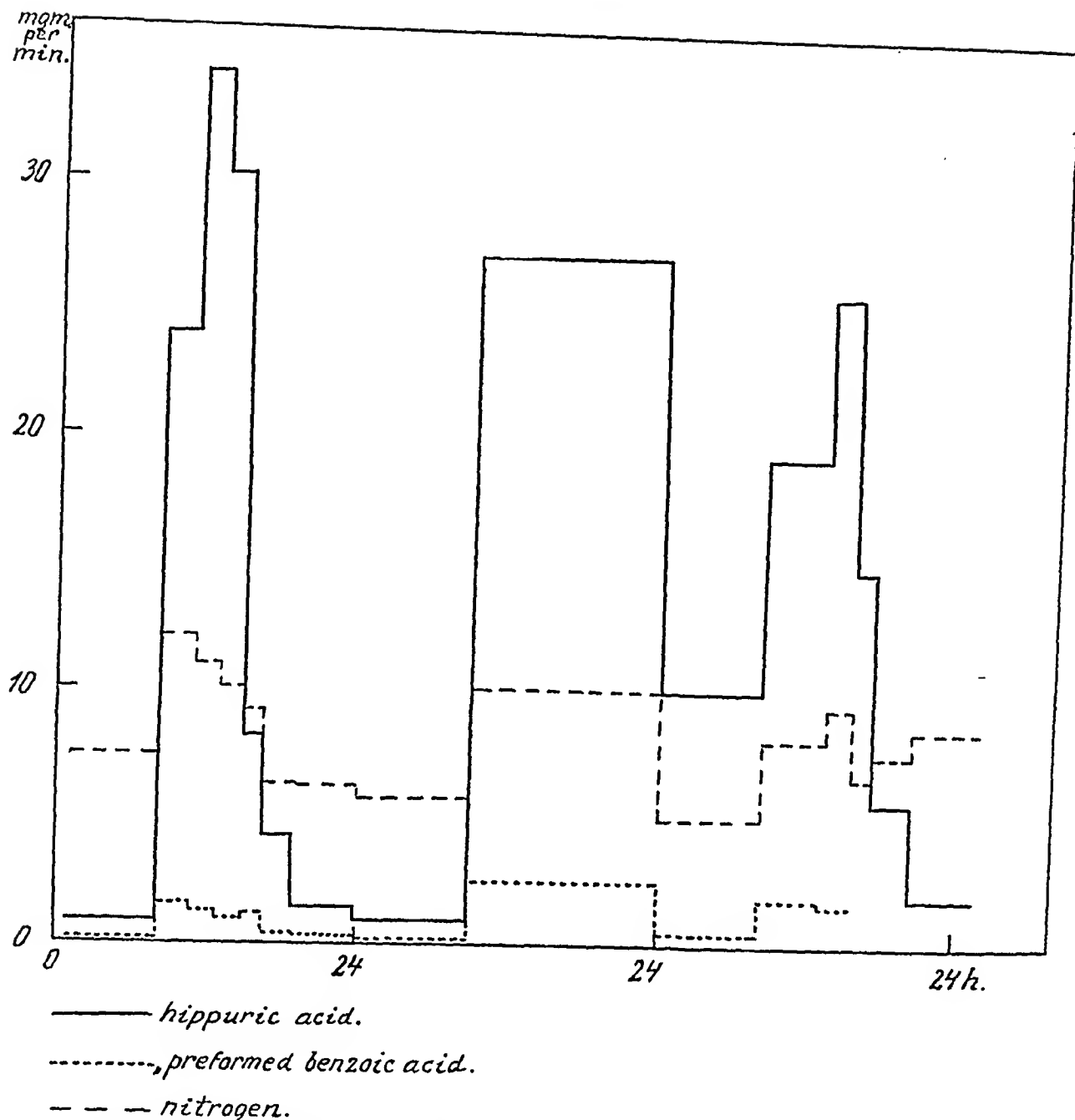


FIG. 1. SYNTHESIS OF HIPPURIC ACID AFTER FEEDING BENZOATE TO A NORMAL PERSON DURING THREE CONSECUTIVE DAYS, TOGETHER WITH NITROGEN EXCRETION.

ing the fluid, the extraction and titration are performed as previously described.

All the analyses were made three times. If one differed essentially from the other two, it was rejected. If they all differed, a new analysis was made.

*Experiment I.* On the first day of the experiment, 10 grams of sodium benzoate were given at 8:20 a.m., and as will be seen, the greater part of it was excreted within 6 hours. The next day, 8 grams were given at 9 and 12 a.m. and at 7 p.m. Although rather large amounts were seen to be excreted during the following night, a new dose of 10 grams on the third day showed that the ability to synthesize hippuric acid was still unimpaired. During the three days of the experiment, 44 grams of sodium

benzoate were taken (corresponding to 39.7 grams of benzoic acid), and in all 38.68 grams of "total benzoic acid" were excreted, equalling 61.8 grams of hippuric acid, while 59.6 grams should have been expected. This corresponds to a glycine synthesis of 21.4 grams.

The elevations in the nitrogen curve are probably due to the nitrogen contained in the hippuric acid, although they are not greater than might have been expected from the food intake during the daytime.

After the ingestion of 10 grams of sodium benzoate dissolved in water, a slight but rather disagreeable itch is felt in the throat, but it soon disappears. Within ten minutes, a sensation of heat, tingling, and pulsation is felt in the head and skin, accompanied by a transitory xanthopsia. These symptoms all subside within an hour. There is no nausea.

TABLE III  
Benzoate feeding to 6 patients with progressive muscular dystrophy

Patient number	Sex	Age	Date	Benzoic acid ingested†	Benzoic acid excreted		Hippuric acid excreted	Glycine calculated		Total nitrogen excreted		Creatinine total	Creatinine total
					Pre-formed	Total		From benzoic acid ingested	From hippuric acid excreted				
		years	1935	grams	grams	grams	mgm. per minute	grams	grams	grams	mgm. per minute	mgm.	mgm.
1	M	16	August 26	0	0.10	1.12	1.24	0	0.69	8.76	6.1	404	555
			August 27	25.4	3.27	20.40	22.60	15.6	12.55	11.40	7.9	486	810
			August 28*	0	0.08	0.34	1.81	0	0.21	2.18	7.3		
2	M	24	August 23	0	0.25	2.00	2.23	0	1.23	10.80	7.5	891	612
			August 24	25.4	4.44	22.80	25.30	15.6	14.00	15.50	10.8	1050	570
			August 25*	0	0.06	0.15	0.78	0	0.89	1.52	5.1	991	370
3	F	10	September 3	0	0.32	0.61	0.68	0	0.38	1.89	1.3		
			September 4	8.5	0.62	8.20	9.10	5.2	5.04	3.11	2.2		
			September 5	0	0.10	0.38	0.42	0	0.23	2.55	1.8		
4	M	34	August 17	0	0.20	0.73	0.81	0	0.45	6.73	4.7	1400	260
			August 18	25.4	3.72	22.70	25.20	15.6	13.95	10.40	7.2	1370	222
			August 19*	0	0.02	0.14	0.75	0	0.86	1.37	4.6	1030	108
5	M	21	April 6	0	0.39	1.77	1.96	0	1.09	10.75	7.5	481	305
			April 7	20.0	3.40	18.62	20.30	12.0	11.45	12.33	8.6	656	702
			April 8	0	0.27	1.02	1.13	0	0.63	9.75	6.8	549	403
6	M	34	October 22	0	0.27	0.98	1.10	0	0.60	7.56	5.3	578	720
			October 23	25.4	1.79	19.20	21.30	15.6	11.80	9.00	6.3	516	1063
			October 24	0	0.55	1.77	1.96	0	1.09	7.00	4.9	559	962

\* Only the urine from the first five hours (7 to 12 a.m.) was collected for determination of benzoic acid.

† Given as sodium benzoate.

*Experiment II.* Table III shows that the patients excrete 80, 90, 97, 90, 93 and 76 per cent respectively of the benzoic acid ingested, thereby producing glycine in amounts varying from 11 to 14 grams, amounts which are fairly comparable with the therapeutic doses of 15 to 30 grams a day.

The nitrogen excretion shows only slight variations, although there seems to be a rise on the day of benzoate feeding.

Since all the patients show essentially the same features in the experiment, we might, as an example, subject Patient Number 2 to a closer examination. On the day of the experiment he excretes 14 grams of glycine, with a nitrogen content of 2.61 grams. If this amount of glycine had been taken from a protein molecule containing four per cent of glycine, while the rest of the molecule had been metabolized, we should have expected the nitrogen content of the urine to rise 25 times 2.61 grams, viz., 65 grams. As the increase is only 4.7 grams, which is but slightly more than is contained in the glycine synthesized, it must be concluded that all the nitrogen of the protein molecules which the organism liberates for this purpose, can be utilized in the synthesis of glycine; in other words, that a true synthesis is going on. This result is in good agreement with McCollum and Hoagland (7), as well as with Linneweh and Linneweh (6).

A study of the excretion of creatine and creatinine does

not lend any support to the view held by Brand and co-workers (1, 3, 5), that creatinuria is lowered after benzoate feeding. The creatinuria shows fluctuations during the three days of the experiment similar to those usual in these patients. In some cases it is higher on the day of benzoate feeding than on the day before and after, in other cases it is lower.

#### CONCLUSIONS

The experiments show that six patients with progressive muscular dystrophy can synthesize almost unlimited amounts of glycine for use in the synthesis of hippuric acid.

From this the conclusion is drawn that progressive muscular dystrophy cannot be caused by a defective synthesis of glycine, and that the beneficial effect of glycine treatment cannot be due to glycine acting as a supplement to the organisms own insufficient production as held by Thomas and coworkers (9).

The experiments showed no reduction of creatinuria when benzoate was fed, so that this ob-

servation does not support the hypothesis that creatine is synthesized from glycine.

#### SUMMARY

1. A critical account is given of experiments showing the synthesis of glycine in patients with progressive muscular dystrophy.

2. The results of benzoate feeding on six patients suffering from progressive muscular dystrophy are recorded together with similar experiments on one normal subject.

3. The experiments show that the patients are capable of synthesizing glycine equal to the usual therapeutic doses.

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# RESULTS OF IMMUNIZATION BY MEANS OF ACTIVE VIRUS OF HUMAN INFLUENZA<sup>1</sup>

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The studies reported here deal with the prevention of "influenza" in a group of individuals immunized by means of intramuscular injections of active<sup>2</sup> human influenza virus in the presence of an oncoming epidemic which occasioned a marked morbidity in the unvaccinated people of the same community.

Shope (1, 2, 3, 4) first reported the successful immunization of hogs against swine influenza by the intramuscular injection of active swine influenza virus; the swine were immunized not only against the mild respiratory disease, "filtrate disease," caused by the virus alone, but also against the severe respiratory disease, true swine influenza, which is caused by the synergistic activity of the swine influenza virus and *Hemophilus influenzae suis*, an organism indistinguishable morphologically and culturally from the non-indol-producing strains of the human Pfeiffer's bacillus. Smith, Andrewes, and Laidlaw (5, 6, 7, 8, 9) and Francis, Magill and Shope (10, 11, 12, 13, 14, 15) demonstrated that ferrets and mice could be infected by the viruses of swine and human influenza and could be immunized against the severe manifestations of the diseases by the subcutaneous injection of active virus. The human influenza virus was more active in its production of cross-neutralizing properties than was the swine influenza virus which they found might or might not produce such cross-neutralization depending apparently upon the number of exposures. They noted that a ferret recovering from the swine influenza virus became partially or completely immune also to the virus of human

influenza and vice versa; the human influenza virus, however, was more active in its production of such a cross-immunity. The immunity in the recovered ferrets remained for approximately three months (8). Neutralizing properties in the sera of ferrets were at a high level following the disease or following active immunization and were still present, but in much reduced amounts, when the animals again became susceptible to the disease (8). The synergistic activity of a virus and a bacterium was not necessary to produce a severe lower respiratory infection in ferrets and mice, although Francis (16) has shown that ferrets harboring pathogenic hemolytic streptococci develop more severe manifestations of lower respiratory infection when influenza virus is injected into the respiratory tract than do the animals in which streptococci are absent. These workers demonstrated that susceptible animals were not infected by injections of active virus except when it was placed directly in the respiratory tract, since in their experiments subcutaneous, intramuscular, intraperitoneal and intravenous injections did not produce the disease.

In view of the successful production of active immunity in lower animals and in view of the prevalence of human influenza due to similar strains of virus in such widely separated places as Australia (17), England, Puerto Rico, New York, Alaska and Philadelphia (14, 18), it appeared essential to study the production of active immunity in human beings with a view towards prevention of the epidemic disease.

## Neutralization tests

In September and October of 1935, several members of the hospital staff and a few hospitalized children who showed only small amounts of neutralizing substances in their sera against the human and swine viruses (PR-8 of Francis and

<sup>1</sup> These studies were assisted by a grant from the Bureau of Animal Industry, United States Department of Agriculture, and by the Lyophile Serum Fund donated by the Board of Managers of the Abington Memorial Hospital.

<sup>2</sup> The term "active" is here applied to virus which is capable of producing the disease.

S-15 of Shope) were injected intramuscularly with both agents in an active state. An increase in the amount of neutralizing substances to the homologous virus in the sera of a number of the

which resembled influenza clinically appeared rather suddenly in and around Philadelphia. Shortly following the advent of the first few cases, immunization with the active viruses of

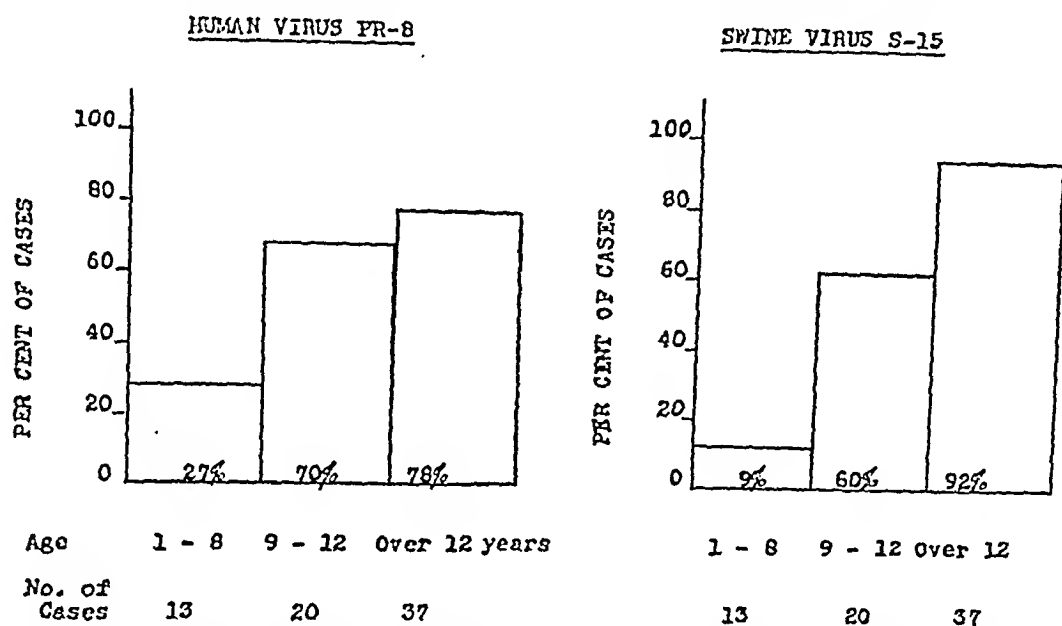


FIG. 1. VIRUS NEUTRALIZING SUBSTANCES BY AGE GROUPS IN UNVACCINATED INDIVIDUALS

individuals was noted, results similar to those reported for experiments with the human influenza virus conducted simultaneously by Francis and Magill (19).

During the fall and early winter of 1935, sera of a large group of individuals over a wide age range were tested for the presence of neutralizing antibodies against the viruses of human and swine influenza. The results of the tests conform in general to those conducted by the workers already mentioned (20, 21), and are shown in Figure 1. In contrast to the results already reported a few of the sera of the children under twelve years of age showed neutralizing properties against the virus of swine influenza S-15 of Shope.<sup>3</sup>

A large number of the sera included in Figure 1 were obtained from a State Colony<sup>4</sup> of approximately eight hundred males, men and boys, near Philadelphia, and it was during the studies of them in February, 1936, that an epidemic

human and swine influenza was initiated in the colony.

#### *Preparation of vaccine*

In the preparation of vaccine for the study of active immunization, the viruses (S-15 of Shope and PR-8 of Francis) were grown both upon chick embryo in modified tissue cultures and in mouse lung. During the early part of the winter of 1935 the PR-8 strain grown upon chick embryo failed to give constant lesions in mice. For this reason and as a temporary expedient, a 10 per cent emulsion of infected mouse lung in normal saline solution was used as a vaccine. This emulsion was filtered through a Berkefeld filter, the filtrate was cultured, and intramuscular injections of 0.5 cc., 1 cc. and 1 cc. were given at intervals of one week. Anesthetized mice were inoculated intranasally with the filtrate immediately preceding and following the intramuscular injection, in order to be certain that active virus was present in the vaccine. The Swiss mice used for the vaccine were from a stock colony, free as far as could be determined from viruses infectious for man, including the virus of choriomeningitis described by Armstrong and Lillie (22) and by

<sup>3</sup> This finding may have been due to the comparatively close contact of this group of children with a herd of swine.

<sup>4</sup> We are indebted to the Department of Institutions and Agencies of the State of New Jersey, and to Dr. Carroll T. Jones, Superintendent of the Colony, for their cooperation in these studies.

Rivers and Scott (23). There was some loss of virus during filtration, and for this reason, as well as for the better control of possible extraneous viruses, we would have preferred chick embryo media, had the growth of PR-8 virus been constant. Experience has shown also that chick embryo tissue is a weak antigen in comparison with the protein of mouse lung. In later studies an alteration of the method of culture on chick embryo insured relatively constant growth of the virus.

#### CLINICAL STUDY

The colony had 17 houses amongst which the inmates were about equally divided. There was a common dining hall and a common amusement hall which insured close contacts of all the inmates. Approximately one-third of the inmates of each house was injected, one-half of this number, namely one-sixth, being injected with active swine virus, the other one-sixth with active human virus.<sup>5</sup> A greater number were given swine vaccine than human vaccine so that in all 110 were injected with human virus and 138 with swine virus; approximately 550 were unvaccinated. Mild local reactions to the vaccine were noted in a few individuals, and in one instance marked localized erythema and tenderness with slight swelling remained for two days at the site of injection. Although those injected were under constant observation, no allergic or generalized reactions were noted. Blood for neutralization tests was collected from the 248 injected individuals before vaccination and again two weeks following the third injection of vaccine. At the same time blood for similar tests was collected from sixty of those not vaccinated. Because the first neutralization tests showed that the undiluted sera of a large part of the colony neutralized both swine and human influenza viruses, further tests were performed with serum diluted as much as 1:80. The neutralizing antibodies in collected serum do not change over a considerable period of time at ice-box temperature, and for this reason it was possible to examine the sera taken

before and after the injections of vaccine at the same time against the same virus suspension. It was found that the dilution of sera with physiological salt solution had a deleterious effect on the viruses, and after trial of several diluents, nutrient broth was selected as the most satisfactory. Healthy white mice<sup>6</sup> were inoculated under ether anesthesia with 10 per cent saline suspensions of either the swine or human strain of virus. The mice were killed when moribund or at the end of the sixth day, and their lungs were removed aseptically and stored in 50 per cent glycerine. A weighed 2 per cent saline suspension of these infected lungs was made and centrifuged at about 1200 r.p.m. for two minutes. The sera were diluted with nutrient broth 1:10 and 1:40 for testing against the human strain, and 1:20 and 1:80 for the testing against the swine strain. The diluted sera were mixed with equivalents of a 2 per cent suspension of infected lung and placed in the ice box at 3 to 4° C. overnight. Each mixture was inoculated intranasally into three mice under ether anesthesia. At the end of the sixth day the mice still living were killed and their lungs examined. The neutralizing effect of a serum of unknown potency was determined by a comparison of the extent of lung lesions in mice receiving the mixture of the virus and the serum in question, with the lesions in the mice receiving the mixture of nutrient broth and virus. The severity of the lung lesions was tabulated as 1, 2, 3, and 4+.<sup>7</sup>

Table I shows a summary of the results of the neutralization tests in the three groups of individuals in the colony. Due to a number of factors, such as contamination and the necessity for repetition, it was not possible to include in this table all of the sera collected. It is to be noted that a significant increase in the amount of neutralizing antibodies occurred only in the indi-

<sup>6</sup> We are indebted to Sharpe and Dohme Company for the majority of mice used in this study.

<sup>7</sup> "1+" Influenzal pneumonia involving up to ¼ of lung at postmortem.

"2+" Influenzal pneumonia involving from ¼ to ½ of lung at postmortem.

"3+" Influenzal pneumonia involving from ½ to ¾ of lung at postmortem.

"4+" Influenzal pneumonia involving from ¾ to all of lung at postmortem.

<sup>5</sup> Shope demonstrated in his experiments on influenza in swine that if as much as one-half of a herd of swine was actively immunized, an epidemic of the disease would not spread readily through the herd; whereas when only one-third of the herd was immunized a more rapid spread of the disease occurred.

viduals injected with the human strain of virus; even in this group only 31 per cent, as calculated, showed such an increase. In the summary of a previous report (24) it was stated that "both the human and the swine vaccinated groups showed an increase in neutralizing substances to their homologous viruses, and to a lesser extent to their heterologous viruses." In the repetition of the tests the use of nutrient broth in place of physiological salt solution as a diluent altered the findings as noted in Table I.

TABLE I  
*Summary of neutralization tests*

Group	Total number of sera tested	Increase		No increase		Decrease	
		Number	Per cent	Number	Per cent	Number	Per cent
AGAINST S-15 SWINE INFLUENZA VIRUS							
Unvaccinated.....	43	8	18.6	35	81.4	0	0
S-15 vaccinated...	107	21	19.6	83	77.6	3	2.8
PR-8 vaccinated..	86	14	16.2	72	83.8	0	0
AGAINST PR-8 HUMAN INFLUENZA VIRUS							
Unvaccinated.....	45	4	8.8	38	84.4	3	6.7
S-15 vaccinated...	112	10	8.9	98	87.5	4	3.5
PR-8 vaccinated..	84	25	31.0	58	69	0	0

#### RESULTS

The epidemic covered a period of approximately two months, beginning in the latter half of February, with the largest number of cases occurring during the fifth week. Although a few cases of the disease occurred previous to vaccination, during the period of the study approximately 25 per cent of the colony developed respiratory infections. These were divided into febrile and afebrile groups. This was possible since all febrile individuals with mouth temperatures of 99.6° F.<sup>8</sup> or over were immediately removed from the houses and placed in bed in an excellent hospital on the colony grounds.

Amongst the febrile group the clinical picture was typical of that associated with epidemics of grippé or influenza with a low leukocyte count of

<sup>8</sup>It was customary in the colony to hospitalize only those with temperatures considered significant, and for this reason the arbitrary figure of 99.6° F. was established.

4000 to 7000 (unless secondary complications occurred), considerable backache, headache and aching in the muscles of the extremities, and certain signs and symptoms of upper and lower respiratory involvement. The afebrile group resembled more closely cases of common cold. There was some overlapping of signs and symptoms in the two groups. The epidemic was considered at an end when admissions to the hospital dropped to one every other day. Table II, showing the com-

TABLE II  
*Summary of clinical results*

Group	Number in group	Febrile cases		Afebrile cases	
		Number	Per cent	Number	Per cent
Unvaccinated.....	550	69	12.5	59	10.7
S-15 vaccinated.....	138	17	12.4	20	14.3
PR-8 vaccinated.....	110	3	2.7	16	14.5

Application of the X<sup>2</sup> test to these data shows that the observed differences in the febrile cases between the PR-8 vaccinated and the unvaccinated groups are significant. The above data differ slightly but not significantly from the preliminary report mentioned (24) owing to a more complete analysis of the case records than was possible at the end of the epidemic when the preliminary report was made.

parative incidence of febrile and afebrile cases in the vaccinated and the unvaccinated groups, includes only cases which occurred after the seventh day from the time of the first vaccination. Other studies have demonstrated the development of immunity during the second week following exposure to the virus (25). The essential point in Table II is the low incidence of the febrile disease in the group vaccinated with the human virus. In only one of the three febrile cases occurring in this group, a boy of 8 (F. C.), did the temperature rise to over 100° F. This boy developed a temperature of 103° and appeared to have typical influenza. That the low incidence might have been due to a nonspecific reaction from the emulsion injected is not probable, since the percentage incidence of the febrile disease in the group vaccinated with swine virus was equal to that of the unvaccinated group. In addition, it is evident that the incidence of afebrile infection in the three groups appears to be affected little if any by vaccination with either virus.

It appears probable that the afebrile groups in-

cluded both the cases of common cold and the cases of mild influenza. On this account a reduction would have been expected in the incidence of afebrile cases amongst the group vaccinated with human virus. The absence of such a reduction may have been due to the fact that the vaccination shifted to the afebrile or mild group a number of cases who without vaccination would have been in the febrile group.

### *Influenza virus as a probable causative agent in the epidemic*

At the height of the epidemic, washings were taken from the noses and throats of three unvaccinated individuals who were hospitalized and from two hospitalized individuals who had received swine virus vaccine. Since we were unable to obtain ferrets at this time, Dr. Francis kindly agreed to pool the washings from the vaccinated and unvaccinated groups respectively and to inject them into two ferrets at the Rockefeller Institute for Medical Research. One of the ferrets died of pneumococcal empyema and no influenza virus was obtained by passage from the other ferret.

It is also possible to determine the presence or absence of the influenza virus as a causative agent of such an epidemic by means of neutralization tests on the sera of unvaccinated individuals who suffered attacks of the disease (12). The serum was collected in the early stages of the disease and three weeks following the attack, and a comparison of the results of neutralization tests was made. Such determinations were made on the sera of a few of the unvaccinated hospitalized individuals in the colony and on the sera of a number of infants and children in the nearby city of Philadelphia who were attacked during the same epidemic and in a similar manner to those in the colony. Amongst the individuals in this entire group who showed no neutralizing antibodies in their serum at the height of the disease, there were found some who had them against the human virus, in a 1:1 mixture of the virus suspension and serum, following convalescence. In one child partial neutralization was found following convalescence, while in the other children there was complete neutralization. The evidence therefore is good that the human influenza virus was

present during the epidemic; that it was the causative agent also appears probable. In the few cases studied which already possessed marked neutralizing properties in their sera, no increase or decrease of such antibodies was noted following convalescence, with the exception of one case which showed a moderate decrease.

The neutralization tests on the sera of these children are shown in Table III. As mentioned

TABLE III

*Neutralization tests on sera taken at height of illness and during convalescence in the youngest group of cases from the epidemic area around the colony*

Case	Age	Highest temperature	Days in hospital	Days of illness previous to first test	Neutralization tests	
					Date	Result
	years	°F.			1936	
T.H.	7	104.0	13	3	March 19	Complete protection
E.D.	3	100.2	21	11	March 18 April 7	Complete protection Partial protection
W.B.	2	103.8	70	17	March 22 March 30	Complete protection Complete protection
L.G.	3	105.0	28	15	March 26 April 4	No protection Incomplete protection
J.J.	1½	105.0	13	7	March 17 March 21	No protection No protection
L.L.	6	100.8	15	3	March 18 April 7	No protection Complete protection
J.P.	4	99.8	4	12	March 30 April 4	No protection Complete protection
E.R.	2	103.4	17	14	March 24 April 7	No protection Complete protection
E.S.	5	105.6	16	21	March 30 April 10	Complete protection Complete protection

in this report and in the reports of other workers, neutralizing properties against the human virus are found in approximately 66 per cent of children at these ages (20). The fact that complete neutralization of the virus was found in a number of the cases during their illness may have been due to the length of illness before admission to the Hospital and therefore before the serum was withdrawn; all of the children were admitted on account of severe complications such as bronchopneumonia following mild upper respiratory disturbances of considerable duration. The length of their illness previous to the first withdrawal of serum for testing is shown also in Table III.

### DISCUSSION

The epidemic described was of moderate severity. That it was considered as influenza from



the clinical standpoint depended chiefly upon the low leukocyte counts and the respiratory symptoms together with aching of the limbs, head, and back. From the laboratory standpoint the appearance of neutralizing antibodies in the sera of unvaccinated children in the epidemic area around the colony during convalescence when none were present in their sera during the illness, places the human influenza virus in all probability as the causative agent. The low incidence of the epidemic disease in the group of individuals vaccinated with the active human influenza virus corroborates the clinical and laboratory evidence. Studies of the nasal and throat washings were not sufficient to furnish significant data.

It is of interest that a large group of individuals who possessed neutralizing properties against the virus of human influenza should still be susceptible to the disease, if the human virus was the causative agent. The findings of Smith, Andrewes, and Laidlaw (8) that ferrets with waning immunity may be infected while still showing neutralizing antibodies in their sera may explain this susceptibility. In addition, it is of interest that vaccination of a large group of individuals with active human influenza virus increased the serum neutralizing antibodies in only 31 per cent, and yet appeared to increase the resistance to infection in practically the entire group injected. That immunity and neutralizing properties are associated but do not in many instances run parallel to each other has been well demonstrated by Shope in his studies of swine influenza virus. However, when such neutralizing antibodies appear for the first time in an individual's serum during convalescence from an infection, the influenza virus in all probability is the responsible factor.

#### SUMMARY AND CONCLUSIONS

1. The neutralizing properties of human sera collected from individuals of different age groups, against the viruses of human and swine influenza are tabulated and compared with the results of other workers.

2. The intramuscular vaccination with active swine and human influenza virus of a group of individuals in a large State Colony, in the presence of an oncoming epidemic of "influenza," is

described. One hundred and ten individuals were vaccinated with human virus and 138 individuals with swine virus, while 550 were not vaccinated.

3. The incidence of febrile and afebrile infection in the colony is tabulated. There was an incidence of 2.7 per cent febrile cases in the group vaccinated with human virus as compared with an incidence of approximately 12.5 per cent in the other two groups. This difference is statistically significant. In a comparison of the incidence of the afebrile, the "common cold" type of infection, in the three groups no significant difference was noted.

4. The presence of the human influenza virus during the epidemic as a probable causative agent was determined by means of neutralization tests on the serum of a number of hospitalized children in the epidemic area who had not been vaccinated. During the height of their illness no neutralizing antibodies against the human virus were found, whereas during convalescence they appeared for the first time. Studies of the nasal and throat washings from the febrile cases were not sufficient to furnish significant data.

5. Additional evidence is furnished suggesting that resistance to infection and neutralizing properties of serum are associated but probably do not always parallel each other.

6. Further studies of a similar nature among larger groups of individuals will be necessary before final conclusions can be drawn concerning the value of immunization against influenza with the active human influenza virus.

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# THE ABSORPTION OF HEXOSES FROM THE UPPER PART OF THE SMALL INTESTINE IN MAN

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The most direct approach to the study of absorption from the gastro-intestinal tract, both in man and in animals, is offered by those methods that make use of the introduction of a known amount of a test substance into the alimentary canal, followed by the estimation of what is left after a definite period of time. Cori (1, 2), working with animals, introduced the substance by stomach tube, killed the animal after a certain time, and determined the amount of the substance still present in the digestive tract at that moment. His technique has been adopted by many other physiologists. The procedure of intestinal intubation with the aid of a rubber tube having a collapsible balloon at its lower end developed by Miller and Abbott (3) offered an opportunity for a direct study of intestinal absorption in man. The present paper gives the results of a study of the absorption of three hexoses: glucose, galactose and levulose in normal adult human subjects. Similar studies, carried out on patients with intestinal disturbances will be reported elsewhere.

## METHODS

A complete description of the tube is given by Miller and Abbott (3) and Owles (4). Their technique has been modified for the present investigation.

*Principles.* Miller and Abbott have recommended the use of a three-lumen tube furnished with two balloons for absorption experiments. They contend that in studying absorption in segments of small intestine that are isolated between two balloons an objective measurement of the process of absorption *per se* is obtained. On the other hand, it seemed to us that under these circumstances a free flow of gastric juice and bile into the segment that is being studied cannot take place, which might interfere with the normal conditions of absorption. Moreover, in working with hypertonic solutions a considerable secretion of fluid takes place from the intestinal wall into the lumen. The accumulation of fluid tends to distend an isolated loop which may give rise to pain and leakage.

For these reasons, we have chosen a tube with two lumina and only one balloon.

The tube is furnished with metal markers at certain points which enable the investigator to ascertain its exact position on the x-ray screen or on an x-ray plate. One of the lumina leads into the collapsible rubber balloon attached at the end of the tube. Once the tip of the tube reaches the jejunum the inflation of the balloon with air to the correct pressure will block off the intestine, so making the duodenum and upper part of the jejunum a cavity closed at the lower end. The second lumen of the tube leads into a number of holes that open above the balloon; through these the test substance is introduced into the intestine. The test substance is thus in contact with the mucous membrane of the duodenum and the upper part of the jejunum between the pylorus and the balloon. It is allowed to remain there for a certain time, after which what is left is recovered by aspiration and washing.

Miller and Abbott (3) observe their subjects under the fluoroscope during the introduction of the tube. We have preferred to introduce the tube first by the routine procedure customary for intubation of the duodenum. The subject swallows the tube quickly until the 55 cm. mark is reached, when the tip of the tube is in the stomach. The subject then lies down on the right side and is instructed to continue swallowing the tube slowly until the tip of the tube has reached the desired position in the jejunum at about 125 to 130 cm. from the teeth. The time required for the passage of the tip of the tube to the desired point varies between 2 and 4 hours. The main delay occurs at the pylorus and the duodeno-jejunal flexure.

The criteria that furnish the indications that the tube has entered the jejunum are: (1) the length of the tube swallowed without kinking by the subject; (2) the appearance of bile on aspiration through the tube; (3) the localization of the pain experienced by the subject on inflation of the balloon. Usually, so long as the balloon is still inside the stomach, the subject feels only a vague sensation; if the balloon is inside the duodenum, most subjects localize the pain on inflation very definitely in the median epigastric region, a few in the right hypochondrium. After the tip of the tube has passed the duodeno-jejunal flexure, the localization becomes much more variable: the pain is experienced in the left hypochondrium, the umbilical region or the lower part of the abdomen, either to the right or left. Without claiming an absolute validity for these subjective localizations

<sup>1</sup> Rockefeller Foundation Fellow, 1935-1936.

(which can never take the place of an x-ray control), we have obtained from them very useful information. The subject is now examined either by fluoroscopy or by exposure of an x-ray plate. By this means the exact position of the tube is definitely ascertained. This simplification saves the subjects much exposure to radiation and it

observed to pass either backward through the pylorus or forward past the balloon (Figure 1). We feel confident therefore that no regurgitation took place during the absorption tests to be described, provided the subjects were quiet, the amount of fluid introduced not too great, and the pressure in the balloon not too high.



FIG. 1. THE TUBE IN SITU

The barium is seen to occupy the duodenum and upper part of the jejunum.

also makes the method available for investigators who have not the equipment necessary for observing subjects continuously under the fluoroscope. Moreover, it makes the intubation applicable to patients who are too ill to stand much handling.

*Controls.* The question of regurgitation into the stomach during the absorption tests required special consideration. In two experiments in which barium sulphate was added to a concentrated glucose solution, the fluid could be seen under the fluoroscope moving up and down and spreading out over the whole of the area between the pylorus and the neck of the balloon. No barium was

In every experiment, some of the stomach contents was aspirated and tested for sugar. If regurgitation had taken place the experiment was discarded. Leakage downward was adequately prevented if the pressure in the balloon was kept above 25 cm. of water. This has been demonstrated by Miller and Abbott (3), Owles (4) and ourselves by the introduction into the gut of non-absorbable substances such as vital red or ferric ammonium citrate that could be recovered quantitatively by aspiration 30 minutes later.

*Subjects.* After the first tests, the subjects were often able to swallow the tube to the desired point entirely by

themselves. All subjects used for this study were adults, volunteers, varying in age between 18 and 60 years. One of the subjects (S) was a woman; the others were men. All were in a healthy state of nutrition. Their weights varied from 120 (S) to 175 pounds (M). Two subjects (T and F) were epileptics, but as far as could be ascertained by routine physical and chemical examinations, were without any sign of "organic disease." No significant differences in absorption that could be attributed to differences in age, sex, or weight of the subjects were discovered. The absorption values in these individuals seemed sufficiently constant within the limits of experimental error to exclude the presence of individual peculiarities.

*Procedure.* By preliminary experiments a period sufficiently long to permit absorption of several grams of sugar was determined. The following standard technique was then adopted, in which the time of the absorption and the time required for aspiration and washing out of the solution were kept constant in every experiment. The fasting subject was instructed to swallow the tube until the tip of the tube appeared to be in the correct location by the criteria described above. An x-ray picture was then made to ascertain the exact position of the tip inside the intestine and to determine the distance between the pylorus and the neck of the balloon. This length was measured from the position inside the intestine of the metal markers on the tube. Since, as will be shown later, the absorption rate varies in direct proportion with the length of intestinal surface area exposed (Table II), all our experiments, when other variables were being studied, were carried out with a constant length (50 cm.) of this "absorption area." Whenever it was not possible to adjust the tube to just this length a correction was used in the calculation of the final results. After x-ray control had given evidence that the tube was in the desired position, the balloon was inflated with air to a pressure of 25 to 40 cm. of water and the maintenance of this pressure controlled. The subject was instructed to lie quietly and avoid coughing.

One hundred cc. of sugar solution were then introduced slowly under constant control of the pressure in the balloon, followed by about 15 cc. of water to force the solution from the dead space in the tube down into the gut. The introduction of the solution took about 5 minutes. All this time the pressure in the balloon was watched and, if necessary, readjusted to the level of 25 to 40 cm. of water.

Exactly half an hour after the beginning of the injection of the solution, the intestinal content was aspirated with an ordinary syringe. The period between introduction and aspiration will be referred to as "absorption time." Aspiration took about 5 minutes, after which a thorough washing followed to insure complete recovery of the material introduced. The washing was carried out by making the subject drink 100 cc. of water four times at 5-minute intervals. This fluid could usually be aspirated from the jejunum 6 to 12 minutes after it had been swallowed. On its way downward it washed out the re-

maining sugar solution. Half an hour after the first withdrawal of the solution from the intestine, Benedict's test for the presence of sugar in the washings was invariably negative. At that time the balloon was deflated and the tube carefully withdrawn.

Intestinal contents and subsequent washings were collected separately and analyzed for sugar both by polarimetry (after precipitation with lead acetate) and by a reduction method (Benedict's). Usually the results agreed within fairly narrow limits. Chlorides, if desired, were determined by Whitehorn's method (5). In many experiments a capillary blood sugar curve was determined along with the absorption test, using the micro method of Folin (6).

## RESULTS

*Influence of concentration on absorption rate of glucose.* In this series of 11 experiments the length of "absorption area" (that is, the distance from the pylorus to the neck of the balloon) was 50 cm. in all tests. The "absorption time" was half an hour. The washing out of the sugar solution also took half an hour. The quantity of solution introduced was always 100 cc. The only variable in the series was therefore the concentration, respectively 5, 7.5, 10, 15, and 20 per cent of glucose. The results are given in Table I. In the

TABLE I

*Influence of concentration on the absorption of glucose by a standard area of 50 cm. length of upper small intestine*

Subject	Date	Quantity of glucose	
		Introduced	Absorbed
		grams	grams
M	March 16, 1936	5.0	4.50
M	March 5, 1936	7.5	6.17
M	November 16, 1935	10.0	7.77
E	April 15, 1935	10.0	9.00
M	November 18, 1935	15.0	8.30
M	December 20, 1935	15.0	7.16
M	December 23, 1935	15.0	7.00
M	December 28, 1935	15.0	7.85
M	November 9, 1935	20.0	7.30
S	December 5, 1935	20.0	8.50
E	November 29, 1935	20.0	7.03
Average of 9 experiments.....		10 to 20	7.77

first two experiments in which the concentration was relatively low, there was a definite increase in the rate of absorption with the concentration. However, in 9 experiments in which the concentration was 10 per cent or over, the quantity of glucose absorbed was independent of the amount

introduced. Apparently the intestine absorbs these concentrated solutions at a uniform rate above which no increase is possible. Under these conditions the amount of glucose absorbed from an area of upper intestine between the pylorus and a point 50 cm. lower down varied from 7.03 to 9.0 grams in the 9 experiments recorded here. The average was 7.77 grams.

In comparing these figures with those obtained by other workers, it should be borne in mind that although the so-called "absorption time" in these experiments was one-half hour, the actual time available for absorption was longer, as some absorption must have gone on during the time when the glucose solution was being washed out. Thus from these observations an idea of the actual amount of glucose absorbed per unit of time can only be derived by approximation. Usually after 40 to 45 minutes the greater part of the sugar solution had been recovered from the intestine. Assuming that all absorption had taken place by that time, one may then estimate that the absorption of glucose from a concentrated solution in a loop of human upper intestine of 50 cm. length goes on at a rate of 7.77 grams per 40 to 45 minutes or 10.36 to 11.65 grams per hour.

TABLE II  
*Influence of absorption area on absorption of glucose*

Subject	Date	Length of absorption area	Quantity of glucose		Absorption calculated per 50 cm. length
			Introduced	Absorbed	
		cm.	grams	grams	grams
S	November 26, 1935	30	10	5.40	9.00
S	December 2, 1935	40	15	7.00	8.40
T	March 12, 1936	45	15	7.73	8.66
M	Average of 4 experiments	50	15	7.71	7.71
F	March 8, 1936	55	15	9.35	8.50
E	April 30, 1936	60	15	9.29	7.74
E	December 13, 1935	70	15	10.20	7.30
Average for 6 subjects...					8.18

*Length of absorption area.* Table II shows the results of a second series of 9 experiments in which absorption time and the quantity of glucose introduced (15 grams) being kept constant, the only variable was the length of duodenum and jejunum that took part in the absorption. In these experiments the length of the absorp-

tion area varied from 40 cm. to 70 cm. In one other experiment, 10 grams of glucose was admitted to a length of 30 cm. of intestine. The results show within the limits of the experimental error a direct proportional relationship between the length of intestine that takes part in the absorption and the quantity of glucose absorbed. If the results are calculated for a standard absorption area of 50 cm. they fall within the limits of variation shown in Table I. The minimum absorption calculated in this way was 7.30 grams, the maximum 9.00 grams, the average value 8.18 grams.

TABLE III  
*Influence of pH on absorption of glucose*

Subject	Date	Quantity of glucose		pH (calculated)
		Introduced	Absorbed	
M	Average of 4 experiments	grams 15	grams 7.71	7
M	March 3, 1936	15	6.86	12
E	March 4, 1936	15	6.03	12
M	March 2, 1936	15	6.79	2
M	April 22, 1936	15	6.17	2

*Influence of pH.* Table III shows the results of a third series of 4 experiments upon the influence of the pH upon the absorption of glucose. All conditions were the same as in the first series of experiments but for the addition of 10 cc. of N/10 normal NaOH or of 10 cc. N/10 normal HCl to 100 cc. of the 15 per cent glucose solution. The results show that in each case the absorption of the glucose solution was somewhat diminished by altering the reaction away from the neutral point. At pH 12 the absorption values were 6.03 and 6.86, respectively, at pH 2, 6.17 and 6.79, compared with the average of 7.71 for control experiments at neutral reaction.

The capacity of the duodenum to buffer its content to a neutral reaction was well brought out by these observations. The fasting content of the upper part of the intestine has a neutral reaction (pH 7.0 to 7.1). The intestinal contents aspirated after half an hour were also neutral, irrespective of whether acid or alkaline glucose solutions had been introduced.

TABLE IV

*Influence of preceding dietary regimen on absorption of glucose*

Subject	Date	Quantity of glucose		Remarks
		Intro-duced	Ab-sorbed	
		grams	grams	
M	November 10, 1935	15	8.30	Vegetarian diet for several years
M	December 28, 1935	15	7.85	After 14 days on mixed diet
E	December 13, 1935	15	7.30	Mixed diet
E	April 26, 1935	15	7.34	After 4 days on ketogenic diet
E	April 30, 1935	15	7.74	After 8 days on ketogenic diet
T	March 12, 1936	15	8.66	After 2 days' starvation
F	August 15, 1936	15	8.50	Mixed diet
F	August 19, 1936	15	9.36	After 4 days' starvation
F	August 29, 1936	15	8.10	After 10 days of diet low in carbohydrate and rich in fat and protein

*Influence of preceding diet and of starvation.* Table IV summarizes the results of a fourth series of 9 experiments undertaken to investigate the possible influence of the nutritional condition of the subject on the absorption rate of the glucose solution. The amount of glucose introduced was always 15 grams in 100 cc. of water, the absorption length 50 cm., the absorption and washing time thirty minutes each. Under these circumstances it appeared that the absorption was essentially the same whether the subjects followed a vegetarian regimen or had taken a diet rich in animal protein and fat. One of the subjects (E) took a ketogenic diet that contained 25 grams of carbohydrate for 8 days, after which time the urine gave a positive reaction for acetone. The absorption test gave a normal result.

The effect of complete starvation was tested on two epileptic subjects (T and F). Patient T starved for 48 hours, and Patient F for 96 hours. The fluid intake during the starvation was not restricted. Both showed a high normal absorption for glucose after this period.

*Nature of sugar.* The effect of the nature of the sugar upon the absorption under standard conditions was next investigated. Table V contains the results of a fifth series of 8 experiments in which *galactose* solutions of various strength were tested. The conditions of the experiments were the same as for those on glucose absorption. The figures in Table V show that above a concentration of 15 per cent the absorption rate is

TABLE V

*Quantities of galactose absorbed by a 50 cm. length of upper small intestine*

Subject	Date	Quantity of galactose	
		Introduced	Absorbed
		grams	grams
M	November 25, 1935	15	9.95
M	November 29, 1935	15	10.66
E	December 31, 1935	15	8.19
S	December 10, 1935	15	9.61
N	February 10, 1936	15	11.00
G	February 14, 1936	15	8.62
M	December 3, 1935	20	8.70
S	December 17, 1935	20	8.88
Average.....		15 to 20	9.45

independent of the concentration. The actual amount of galactose absorbed appeared to be larger in the same unit of time than that of glucose, the average value being 9.45 grams, varying from a minimum of 8.19 to a maximum of 11.0 under the standard conditions. An approximation of the absorption per unit of time based on the same principles as used above for glucose would lead to an estimate of 12.60 to 14.17 grams per hour as the rate of absorption of galactose from a concentrated solution in a loop of intestine of 50 cm. length.

TABLE VI

*Quantities of levulose absorbed by a 50 cm. length of upper small intestine*

Subject	Date	Quantity of levulose	
		Introduced	Absorbed
		grams	grams
M	December 24, 1935	6	4.86
E	January 8, 1936	7	5.77
M	December 16, 1935	8	5.46
S	January 13, 1936	8	4.37
M	December 11, 1935	10	4.74
S	January 21, 1936	10	5.42
E	January 22, 1936	10	5.79
Average.....		6 to 10	5.20

Table VI shows the results of a sixth series of 7 experiments upon the absorption of *levulose*. The absorption rate appears to be constant for solutions above 6 per cent in concentration. The absorption of levulose is definitely slower than that of glucose or galactose, varying from 4.37 to 5.79 grams under standard conditions. The av-



erage value was 5.20 grams. Approximation would yield an absorption rate of 6.93 to 7.80 grams of levulose per hour from a 50 cm. length of intestine.

It will be noted that the maximum absorption rate in the case of levulose occurred at a lower concentration (6 per cent) than that of glucose (10 per cent). Apparently the critical level above which the absorption rate becomes uniform is not determined by the osmotic concentration and may be a characteristic property of every individual sugar.

*Influence of the absorption time.* In a seventh series of 4 experiments, all carried out with galactose on the same subject, the "absorption time" was varied to respectively, 0, 15, 30, and 45 minutes with otherwise standard conditions. The washing time was kept constant at one-half hour in all instances. The results are shown in Table VII. As could be expected, a direct, almost

TABLE VII  
*Influence of "absorption time" upon absorption of galactose*

Subject	Date	Quantity of galactose		Absorption time
		Introduced	Absorbed	
		grams	grams	minutes
E	March 11, 1936	15	2.41	0
E	February 19, 1936	15	5.73	15
E	December 31, 1935	15	8.19	30
E	December 18, 1935	15	13.00	45

linear relationship was found between the quantity absorbed and the time allowed for absorption. In the experiment in which the absorption time was "zero," the aspiration of the solution was started immediately after it had been introduced. All the absorption that took place must have occurred, therefore, during the injection of the solution (5 minutes) and the "washing time" (30 minutes).

This series of experiments also furnishes data from which a more exact idea of the absolute value for the absorption rate of galactose can be obtained. By deducting the figure for "0" minute absorption time from that for the 15-minute period, etc., values for the quantity absorbed during exactly 15 and 30 minutes can be obtained. These values vary from 2.46 to 4.81

grams, with an average of 3.44 grams per 15 minutes or 13.76 grams per hour. This agrees fairly well with the values of 12.60 to 14.17 that have been arrived at by approximation.

*Osmotic relationships.* The quantity of fluid recovered one-half hour after the introduction of 100 cc. of the concentrated sugar solution into the intestine was always larger than the amount introduced and varied between 140 and 250 cc., with an average of 200 cc. Apparently, while sugar was being absorbed, fluid had moved into the lumen of the intestine. Though in part probably derived from the small intestinal wall, bile, pancreatic juice, and gastric juice may have flowed into the intestine and contributed to the dilution of the test solution. During the next half hour, while the subjects were drinking a total of 400 cc. of water, another volume of fluid was collected. The washings varied in volume from 200 to 400 cc., dependent on the amount of water that was retained inside the stomach.

The concentration of glucose found in the intestinal contents after half an hour varied from 2.11 to 3.24 grams per 100 cc., with an average of 2.50 per cent. The concentration of glucose was apparently reduced to about half its isotonic concentration (5.4 per cent). This may seem strange at first, as one would not expect a dilution below isotonicity. It should be remembered, however, that the normal duodeno-jejunal juice contains chloride in almost the same concentration as the blood plasma (in our experience between 279 and 475 mgm. per 100 cc.; according to Karr and Abbott (7) between 178 and 528 mgm. per 100 cc.). The chlorides, together with the bicarbonate, maintain the normal osmotic pressure of the intestinal juice. The dilution of a hypertonic glucose solution inside the intestine will go on, therefore, until the concentration of glucose plus the concentration of the other constituents together make up isotonicity. That this holds true was brought out by determination of the chloride in the intestinal content. In the fluid aspirated one-half hour after the introduction of the glucose solution, the chloride concentration was markedly lower than before the introduction of the glucose. The values varied between 212 and 287 mgm. per 100 cc. (Table VIII). Moreover, there was an almost complete inversely proportional relationship between the glucose and

TABLE VIII

*Concentration of glucose and chloride in intestinal content 30 minutes after introduction of 100 cc. 15 per cent glucose solution*

Subject	Date	Glucose	Chloride
	1936	grams per 100 cc.	mgm. per 100 cc.
E	September 29	3.24	212
F	April 23	3.14	219
O	September 25	2.57	229
E	April 27	2.52	224
M	May 19	2.48	228
E	April 30	2.26	229
S	April 28	2.26	243
F	August 19	2.22	287
F	August 14	2.14	287
F	May 12	2.11	271
Average.....		2.49	243

chloride concentration, chlorides being higher in the fluids with the lower glucose content.

If we express both glucose and chloride concentration in milliequivalents and assume that all the cations present are monovalent, we can calculate the sum of the osmotic concentrations of glucose, the chlorides and the cations together. For this purpose we simply multiply the chloride concentration by 2, and add this to the glucose concentration. Table IX shows the figures ob-

TABLE IX

*Glucose and chloride concentration and their combined osmotic pressure in intestinal content at end of absorption period*

Subject	Date	Glucose concentration	Chloride concentration	Combined osmotic pressure of glucose, chlorides and their monovalent cations
	1936	m.eq. per 100 cc.	m.eq. per 100 cc.	"milliosmoles" per 100 cc.
E	September 29	18.00	5.99	29.98
F	April 23	17.45	6.16	29.77
O	September 25	14.28	6.45	27.18
E	April 27	14.00	6.31	26.62
M	May 19	13.78	6.42	26.60
E	April 30	12.56	6.46	25.48
S	April 28	12.56	6.85	26.26
F	August 19	12.34	8.09	28.52
F	August 14	11.89	8.09	28.07
F	May 12	11.72	7.64	27.00
Average.....		13.86	6.84	27.55

tained in this manner. The calculated osmotic pressure in this table is expressed as "milliosmoles" per 100 cc. There is some variation in the figures but on the whole the total concentration of glucose, plus chloride, plus cation, seems to be

fairly constant, varying between 25.48 and 29.77, with an average of 27.29 milliosmoles per 100 cc. We do not know how much carbonate was present in the intestinal content, but this can only have been of small importance (Karr and Abbott (7)). If we compare our calculated osmotic concentration with the normal osmotic concentration of blood plasma (which is about 30 milliosmoles per 100 cc.) it may be safely concluded that half an hour after the introduction of a concentrated solution of glucose the human small intestine has reestablished osmotic equilibrium between its content and the blood plasma.

*Blood sugar curves.* In a number of tests capillary blood sugar values were determined while glucose, galactose, or levulose was being absorbed. Not all the values obtained will be given here. Table X shows only the maximum, min-

TABLE X

*Blood sugar values in milligrams per 100 cc. during the absorption of hexose from the upper part of the small intestine*

Sugar	Blood sugar	Fast-ing	Time after introduction of 100 cc. of solution					
			$\frac{1}{2}$ hour	$\frac{1}{2}$ hour	$\frac{1}{2}$ hour	1 hour	1½ hours	1½ hours
Glucose (11 curves)	Highest	119	153	161	173	188	125	117
	Lowest	99	101	115	118	102	112	108
	Average	107	122	131	134	127	117	111
Galactose (7 curves)	Highest	118	151	152	154	151	125	120
	Lowest	105	108	115	110	101	109	104
	Average	108	120	132	130	122	117	108
Levulose (5 curves)	Highest	112	117	125	121	116	118	107
	Lowest	101	105	109	106	103	100	100
	Average	106	113	114	112	110	107	103

imum, and average values that were found fast-ing and at successive intervals of one-quarter hour after the introduction of the sugar solution.

(a) *Glucose.* In almost all instances when glucose was given the blood sugar showed a definite rise after one-quarter hour, reached a peak after one-half, three-quarters or one hour, then dropped rather steeply and reached its original value again after between one and one-half and one and three-quarter hours. The height of the peak varied between 119 mgm. per 100 cc., and 188 mgm. per 100 cc. The highest point of the average curve was 134 mgm. per 100 cc. after three-quarters of an hour. There was no definite

relationship between the amount of glucose introduced and the height to which the blood sugar rose. It is noteworthy that although the withdrawal of the glucose solution from the intestine was started after one-half hour, the blood sugar level in many instances continued to rise and dropped only after one hour. Glycosuria during the test did not occur in any instance.

(b) *Galactose*. The blood sugar curves after administration of galactose through the tube did not differ materially from those when glucose was being absorbed. Apparently, although the absorption rate of galactose is greater than that of glucose the storage mechanism of the body takes care of the quantity absorbed under the conditions of our experiment. A slight galactosuria occurred in five instances.

(c) *Levulose*. The "glycemic" effect of this sugar was decidedly less than that of glucose or galactose. The highest blood sugar value obtained with levulose was only 125 mgm. per 100 cc. (expressed as glucose). Whether this smaller rise is caused by the slower absorption rate only or whether the tissues remove levulose from the blood at a higher speed than the other sugars cannot be decided here. No levulose appeared in the urine of any of the subjects.

#### DISCUSSION

The present status of our knowledge of the absorption of carbohydrates has been reviewed recently by Pierce (8). Only those points bearing directly on the results obtained in this study need therefore be discussed here.

The fundamental work of Cori (1) carried out in rats has definitely established that the absorption of a simple sugar from the intestinal tract is independent of the quantity of sugar present in the alimentary canal. It should be remembered, however, that this "law" was found by introducing concentrated solutions into the animals. The validity of Cori's law has been confirmed by Cori, Cori and Goltz (9), Holtz and Schreiber (10), Magee (11), Trimble, Carey and Maddock (12), and Verzár (13). Trimble and Maddock (14), working with dogs, made the important observation that Cori's law also applies when the glucose solution is introduced directly into the duodenum. Other investigators (15, 16)

failed to confirm Cori's observations in detail but their results are in substantial agreement with his findings. There still exists some disagreement between investigators whether the absorption rate should best be expressed per unit of intestinal surface (16) or per kilogram of body weight (1, 12).

Our own results confirm Cori's law for the absorption of concentrated sugar solutions from the human small intestine. The results did not warrant a calculation of the absorption rate per kilogram body weight. The area of intestine exposed to the sugar solution appeared to be of paramount importance for the amount of sugar absorbed. The absorption rate per unit of time was constant for a given intestinal length which was assumed to be a relative measure of the surface area.

The absorption of comparatively *dilute* sugar solutions has been studied in animals by Nagano (17), Hewitt (18), and London and Polowzowa (19), who found that the absorption rate increased with the concentration. Abbott, Karr and Miller (20) have shown this to be equally true for the human small intestine. The few experiments undertaken by us with glucose solutions of low concentrations (Table I) confirm their observations. This does not contradict the work of Cori on the absorption of *concentrated* solutions. Our results brought out very definitely that above a certain concentration, which seems to be different for various sugars, the absorption proceeds at a uniform rate.

London and Polowzowa (19), and Magee and Reid (21), both working with dogs, found that a maximum absorption rate for glucose established itself at concentrations of 11.5 and 13 per cent, respectively. In our experiments the absorption rate became maximal at concentrations of 10 per cent or over.

The literature contains a few conflicting reports on the influence of the pH on the absorption rate of glucose (22, 23). From our data we cannot offer an adequate explanation for the slowing down of the absorption rate by the addition of acid or alkali to the glucose solution, as observed by us. The effect need not be specific but might be caused solely by the increase in osmotic concentration of the intestinal content (24). Apparently a neutral reaction affords optimum con-

ditions for the absorption of glucose in the intestine, but this does not necessarily hold true for other substances. It appears that the intestine possesses an efficient mechanism for the protection of the reaction of its content, a point which has been extensively studied by Karr and Abbott (7) and Stevens (25).

Various workers have shown that the intestine absorbs different sugars at a different rate. According to Cori, the proportion of the average absorption rates of galactose, glucose, and levulose (glucose being taken as 100) in the rat can be expressed as 110:100:43. Our results yield for this relationship 122:100:67, which, considering the experimental error in both animal and human experiments, may be called a satisfactory agreement. Cori (1, 2), Wildbrandt and Laszt (26), Verzár (13), and Westenbrink (27) have rightly concluded that since every sugar has its individual absorption rate the process of absorption in the intestine, even of such simple compounds, cannot be a purely diffusion phenomenon, but must be an active cellular process inside the epithelium of the gut. Whatever the nature of this physiological process of sugar absorption may be (28, 29, 30, 31), it appears to be of the same nature both in animals and in man.

A comparison of the actual absorption rates in animals and in man seems hardly warranted because of the different way in which the studies have been conducted. In the animal, the substance is introduced either into the stomach or into the duodenum and then allowed to spread out over all the available intestinal area where the peristalsis will carry it. In our experiments the absorption was limited to a closed loop of intestine of 50 cm. in length. Even so, it is interesting to note that the approximate average absorption rates of glucose per hour when calculated per kilogram body weight were:

For the rat.....	2.00 grams (1, 9, 13, 16)
For the dog.....	1.00 gram (14)
For the human.....	0.18 gram (present study)

This furnishes another example of the higher speed at which the living processes seem to go on in the smaller animals.

Most of the blood sugar curves obtained in our experiments were only slightly lower than those observed in the capillary blood during the

first hour of an ordinary glucose tolerance test, when 50 to 100 grams are given by mouth to a normal individual. We know, however, that of this large quantity only a limited portion at a time is admitted by the pylorus to the upper part of the intestine. As all other factors influencing the blood sugar curve seem to be the same in the oral and intestinal administration of the sugar, it may be concluded that even if 50 to 100 grams of glucose are given by mouth, the actual absorption is only slightly more than when 10 to 20 grams are fed into 50 cm. of the upper part of the intestine. We have estimated the approximate rate of absorption in our experiments as 10.5 to 12 grams per hour. From the study of the rise in blood sugar during this absorption, we would venture to suggest that even when much larger quantities of glucose are given by mouth the absorption rate is only slightly more than this figure. This would imply that the time for the absorption of a glucose test meal of 50 grams would be 4 hours or somewhat less. We have little doubt that if 50 grams of glucose were introduced directly into the whole of the intestine the absorption would go on at a higher rate and the blood sugar would rise to higher levels (32). Given by mouth, however, the pylorus takes care that the absorption does not take place at a higher speed than the storage mechanism can keep up with (10).

# SUMMARY

The absorption of glucose, galactose, and levulose from the upper part of the human small intestine has been studied by a simplified method of intestinal intubation. The results show that a given length of the human small intestine during a constant interval absorbs a constant amount of a simple sugar from a concentrated solution. Under these conditions, the amount of sugar absorbed is independent of the concentration above a certain level. The amount of sugar absorbed increases with an increase in intestinal surface. The quantity absorbed is proportional to the time allowed for absorption. The addition of dilute hydrochloric acid or sodium hydroxide to the glucose solution diminishes the amount of sugar absorbed. No influence of the immediately preceding diet or of starvation on the absorption rate could be detected.

Glucose, galactose, and levulose have their own individual absorption rates which averaged, under the conditions of our experiments, 7.77, 9.45, and 5.20 grams, respectively, the relation being 100:122:67. During the absorption the concentrated sugar solution inside the intestine is rapidly diluted so that after one-half hour the total osmotic concentration of the intestinal contents equals that of the blood plasma.

Blood sugar values during the absorption of the glucose, galactose, and levulose from the intestine were determined. The curves obtained indicate similar effects for glucose and galactose but a less effect upon blood sugar for levulose than for glucose.

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# GALACTOSE TOLERANCE IN HYPERTHYROIDISM<sup>1,2</sup>

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Impaired tolerance to dextrose is found in about one-half of patients with hyperthyroidism. Since the cause of reduced sugar tolerance in the hyperthyroid state remains unsettled, we endeavored to obtain more information on this subject by studying the response of patients with this disease to galactose.

## METHOD

Forty grams of galactose, dissolved in 400 cc. of water flavored with lemon juice, were administered by mouth to subjects who had fasted for 14 hours. Specimens of blood were obtained from the cubital vein before, and 5, 15, and 30 minutes after, administration of galactose. The dextrose and galactose fractions of these blood samples were determined separately by Raymond and Blanco's modification (1) of the method of Somogyi.

This experiment was performed on twenty-one normal individuals, twenty-six patients with hyperthyroidism, fourteen patients with diabetes, and five patients with miscellaneous diseases of the liver. The experiment was repeated on eight patients after thyroidectomy, and in one case after a period of medical treatment during which marked amelioration of symptoms took place.

## RESULTS

From Table I, it is seen that there was only occasionally galactose in the blood of normal persons at the 5-minute period. Thereafter, the average galactose curve rose to 15 mgm. per cent at the 30-minute period (see Figure 1). The maximum normal galactose reading was 26 mgm. per cent. Most patients with hyperthyroidism had galactose in the blood at the 5-minute period. Galactose curves considerably higher than normal

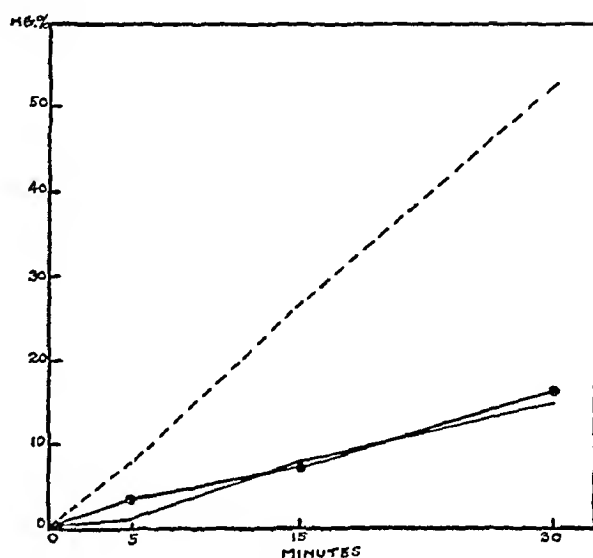


FIG. 1. GALACTOSE FRACTION OF THE BLOOD OF 21 NORMAL SUBJECTS (SOLID LINE), 26 PATIENTS WITH HYPERTHYROIDISM (BROKEN LINE), AND 14 PATIENTS WITH DIABETES MELLITUS (SOLID LINE WITH DISCS), AFTER ORAL ADMINISTRATION OF GALACTOSE.

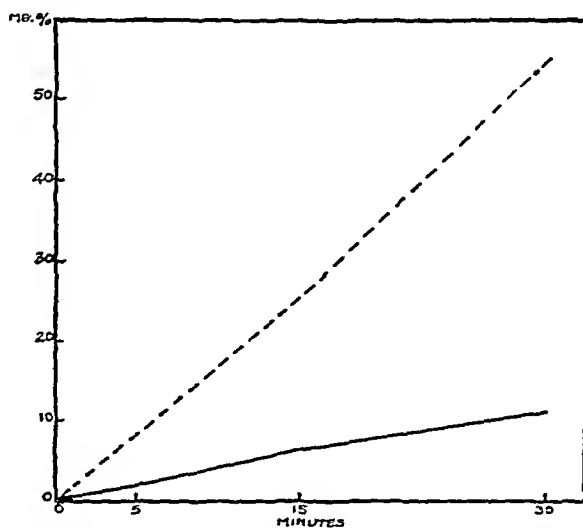


FIG. 2. GALACTOSE FRACTION OF THE BLOOD FOLLOWING INGESTION OF GALACTOSE IN 8 PATIENTS WITH HYPERTHYROIDISM BEFORE (BROKEN LINE), AND AFTER THYROIDECTOMY (SOLID LINE).

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<sup>2</sup> Read by title at the meeting of the American Society for Clinical Investigation, Atlantic City, May 6, 1935.



TABLE I  
*Normal individuals*

Subject number	Galactose tolerance		
	5 minutes	15 minutes	30 minutes
	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
1	0	0	18
2	0	0	1
3	3	4	3
4	0	8	14
5	0	8	20
6	1	9	23
7		9	4
8	0		19
9		14	18
10	0	26	25
11	4	13	8
12	0	14	26
13	1	5	20
14	0	4	4
15	0	13	23
16	3	11	
17	0	9	23
18	4	9	23
19		0	11
20	0	4	18
21		0	4
Average	0.9	8.0	15.2

were the rule (Table II). Only two of these patients had maximum galactose readings below 30 mgm. per cent. Following thyroidectomy a marked increase in tolerance to galactose was observed in patients with hyperthyroidism (Figure 2).

The patients in the diabetic group with two exceptions exhibited normal galactose curves (Table III). As was expected, the galactose fraction of most patients with hepatic disorders indicated diminished tolerance to this sugar.

Changes in the dextrose fraction of the blood, contrary to our original impression (2), were proven by further work not to be sufficiently decisive to warrant reporting.

#### COMMENT

Two explanations of the abnormally high galactose curve in the blood of patients suffering from hyperthyroidism occur to us. Accelerated intestinal absorption of galactose may account for the presence of larger amounts of this sugar in the blood. This possibility is suggested by the earlier appearance of galactose in the blood of patients with hyperthyroidism. We are at present testing

TABLE II  
*Patients with hyperthyroidism*

Subject number	Basal metabolic rate	Galactose tolerance		
		5 minutes	15 minutes	30 minutes
	<i>per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
1	+ 60	12	65	91
2	+ 60		30	65
3	+ 64	4	21	42
3a		5	7	
4	+ 46	0	28	59
4a	- 27	0	0	0
5	+ 40	0	18	51
6	+ 22	12	12	30
6a	- 32	5	9	7
7	+ 32	18	35	40
8	+ 30	5		78
9	+ 46	5	10	31
10	+ 47	9	27	81
10a	- 7	0	5	12
11	+ 56	0	3	23
12	+ 66	17	26	35
12a		3	3	0
13	+ 36	13	18	31
13a	- 17	1	23	39
14	+ 13	3	26	49
15	+ 61	7	35	68
15m	+ 30	4	21	27
15a	- 3	0	5	0
16	+ 45	9	23	65
17	+ 39	5	38	94
17a		1	1	33
18	+ 62	7	7	21
19	+ 58	5		73
20	+ 65	20	29	61
21	+ 59	10	27	41
22	+ 50	14	27	64
23	+ 65	9	45	51
24	+ 70	5	45	40
25	+ 22	7	29	36
26	+ 48	3	27	54
Average *		8.0	27.1	52.8

*a* = after thyroidectomy.

*m* = after medical treatment.

\* Curves obtained after thyroidectomy were excluded from this average.

this hypothesis by studies of the rate of intestinal absorption in animals rendered hyperthyroid.

The second explanation is reduced capacity to utilize galactose, probably due to hepatic injury. Hepatic insufficiency in hyperthyroidism, as indicated by liver function tests, has been observed by ourselves (3) and was also reported by Youmans and Warfield (4) and by Lichtman (5). In addition, Kerr and Rusk (6), and more recently, Weller (7) and Beaver and Pemberton (8) described hepatic lesions in hyperthyroidism which could account for functional impairment of the liver. The reduced ability to handle galactose in hyperthyroidism is evidently not dependent

TABLE III  
*Patients with diabetes mellitus*

Subject number	Galactose tolerance		
	5 minutes	15 minutes	30 minutes
	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
1	0	5	21
2	3	30	77
3	5	20	29
4		7	7
5	0	0	4
6	0	4	9
7	0	8	20
8	0	7	0
9	3	0	0
10	3	7	0
11	23	33	23
12	0	5	14
13	5	0	17
14	4	0	16
Average	3.5	7.6	16.7

upon the low glycogen content of the liver characteristic of this condition, because almost all patients with diabetes who, as a rule, also have a low hepatic glycogen, exhibited normal galactose curves.

The changes in galactose tolerance were often not proportionate to the severity of hyperthyroidism, as judged by any one criterion. This is not remarkable when one considers that the basal metabolic rate and other manifestations of hyperthyroidism are also not necessarily of corresponding severity. In our small group of patients with hyperthyroidism, reduction of galactose tolerance was observed with sufficient constancy to permit it to be compared with the basal metabolic rate as a diagnostic aid. Of twenty-six patients with clinical hyperthyroidism, all but two had a galactose concentration in the blood of 30 mgm. per cent or over. In the same group one patient had a basal metabolic rate of only plus 13 per cent. Another patient was referred to us for hyperthyroidism with a basal metabolic rate of plus 26 per cent and plus 28 per cent respectively on two occasions. He had normal galactose tolerance

and was found to suffer not from hyperthyroidism but from rheumatic heart disease.

At present we are studying a larger series of patients to determine the usefulness of this procedure in the differential diagnosis of hyperthyroidism.

#### SUMMARY

1. The curve of galactose in the blood, after oral administration of this sugar, is considerably higher in patients with hyperthyroidism than in normal or diabetic subjects.

2. Thyroidectomy in most instances restores normal tolerance to galactose.

3. Accelerated intestinal absorption or impaired utilization of galactose by the liver in hyperthyroidism probably accounts for this phenomenon.

4. Reduced tolerance to galactose is such a consistent finding in hyperthyroidism that it may prove to be of value in differential diagnosis.

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# STUDIES OF AN URTICARIAL RESPONSE TO BLUE AND VIOLET LIGHT IN MAN

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A case of *urticaria solare* being available for study, it has seemed wise to determine as many of the characteristics of the photo-response as feasible, in the hope that these might ultimately shed light on the obscure etiology of this rare but distressing disease. Preliminary studies of this case by Blum, Allington and West (1) have shown that the individual is sensitive to wavelengths included in the spectral region between 3900 and 5300 Å, i.e., in the blue and violet parts of the visible spectrum, radiation from which region of the spectrum elicits no response in normal individuals. The *urticaria solare* individual responds to short periods of this kind of radiation with an erythema restricted to the irradiated area, followed by an edematous wheal over the same area with spreading erythematous flare when the irradiation is sufficiently intense or prolonged; this response has thus the characteristics of the "triple response" as described by Lewis (2), and we may make the tentative assumption that it occurs as the result of the elaboration of a histamine-like "H" substance, further evidence for which will be presented in the course of this paper. The response disappears in a few hours, leaving no trace. Ultraviolet radiation shorter than 3200 Å brings forth the same delayed erythema followed by pigmentation that is produced in the normal individual, but no traces of the urticarial reaction characteristic of the response to blue and violet light.

## CLINICAL ACCOUNT

As a clinical report of this case has not been published, the following brief account is included.

The patient, a white male, age 21, associates the first appearance of abnormal sensitivity to light with a bee sting on the left malar area which occurred May 18, 1934. Severe pain and swelling of the face which subsided in a day or two followed the sting. The sensitivity to light was noticed shortly after.

Previous history reveals no allergic or urticarial dis-

orders. Prior to the onset of the present difficulty his skin had always responded normally to exposure to light. There is no family history of allergy or light sensitivity.

Physical examination shows a well developed male of average height and weight. He is a brunette, but the skin is pale from avoidance of light. He is suffering from a moderately severe indolent papular type of acne vulgaris, and presents scattered areas of tinea versicolor over the upper trunk. There are no other abnormalities. The skin reacts normally to heat and cold. There is no dermatographism.

## Laboratory findings

**Blood.** Numerous counts have shown an average of about 5,000,000 erythrocytes and 7,000 leukocytes per cu. mm. There is an increase in small lymphocytes up to about 50 per cent, otherwise the differential count is normal. Hemoglobin determination, made with a photometer, based on the Newcomer standard, was 85 per cent. Spectroscopic examination of the serum for abnormal pigments negative.

**Urine.** Routine examination and examination for porphyrins were negative on several occasions.

**Fasting blood sugar**—100 mgm. per 100 cc.

**Fasting blood uric acid**—3.3 mgm. per 100 cc.

**Wassermann**—negative.

**Gastric analysis.** *Alcohol test meal.*

Time (minutes):	fasting	15	30	45	75	90	105	120
Free HCl	0	0	0	0	0	0	2.4	8.0
Total acidity	4.2	9.6	13.7	14.3	13.3	10.2	14.0	15.0

Scratch tests for a large variety of food, pollen, and epidermal extracts showed no reactions.

**Basal metabolic rate**—minus 14.

## METHODS

**General method.** The arrangement of the apparatus used in the following experiments is diagrammed in a general way in Figure 1. It consists of a 500-watt projection type Mazda lamp *A* placed at a given distance from an opaque screen *B*, which has a circular opening *S* about 2 cm. in diameter through which an area of the skin may be irradiated. The screen is so fixed that the skin of any desired part of the body may be firmly pressed against it without disturbing the arrangement of the apparatus; thus the distance between *B* and *A* is established and reproducible through-

<sup>1</sup> Assisted by a grant from the research funds of the University of California.

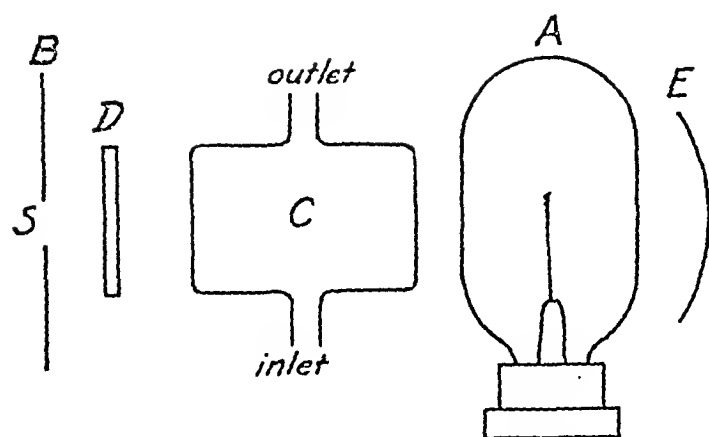


FIG. 1. ARRANGEMENT OF APPARATUS

*A*, 500-watt projection type Mazda lamp; *B*, opaque screen with circular opening *S*; *C*, water filter; *D*, glass color filter; *E*, concave mirror.

out a given experiment. *C* is a water filter for removing infra-red radiation; it consists of a pyrex cylinder 10 cm. in length and 5 cm. in diameter with inlet and outlet tubes so that a current of water may be passed through it to prevent excessive heating of the apparatus. *D* indicates the position at which glass filters may be introduced to obtain restricted wavelength regions. *E* is a concave mirror used in some experiments to concentrate the light rays. The distances between the various elements of the apparatus were altered to meet the requirements for any particular experiment, and in some cases one or more of the elements was removed, as will be explained in the description of the different procedures.

The sensitivity of the skin was measured in terms of the time of irradiation required to produce a just observable erythral response, the *threshold time*; the justification of this criterion will appear as the various experiments are discussed. The measurement was made in the following way. The skin was placed against the screen *B*, and irradiated through the opening *S* for a measured number of seconds. The skin was then moved away from the screen and observed for the appearance of erythema on the irradiated area. Most of the experiments were carried out at room temperature and under these conditions it was found that if erythema did not appear within fifteen minutes after the irradiation ceased, none was ever observed. The procedure was repeated on other areas of skin using different periods of irradiation, the shortest period which would just produce an erythral response being

taken as the *threshold time*. It was found that this *threshold time* could be determined with an accuracy better than ten per cent in most cases but that it was safer to make no attempt to reduce this error. It is quite easy to make the decision as to whether an erythema has appeared or not, when no greater accuracy is attempted.

The 500-watt projection-type Mazda lamps used as sources burn at a higher color temperature than most incandescent lamps, and consequently have a greater proportion of their emission in the blue-violet region in which we are interested. The shape of the emission curve, being very nearly that of a theoretical black body, can be readily calculated from Wien's equation, if the color temperature is known (see Harrison (3)). Figure 2 shows emission curves for one of the

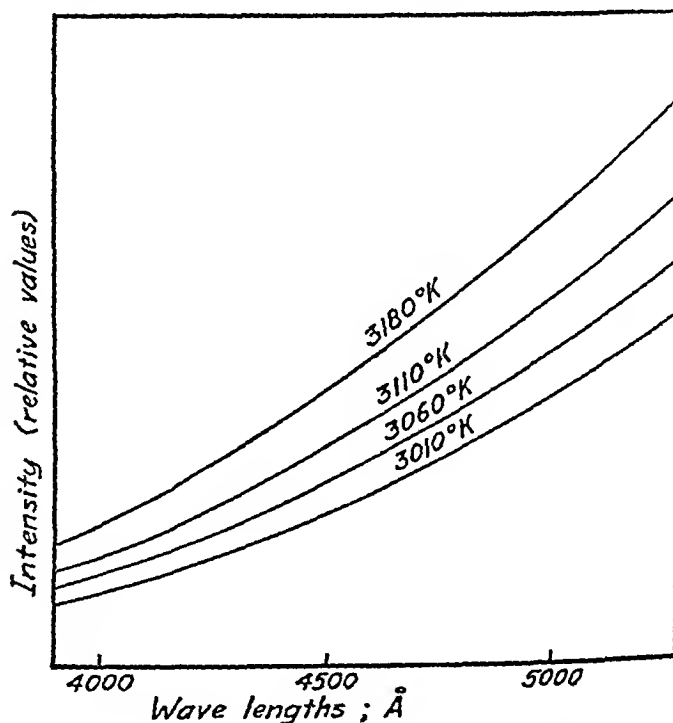


FIG. 2. SPECTRAL DISTRIBUTION OF EMISSION FOR A BLACK BODY AT DIFFERENT TEMPERATURES.

The temperatures are the color temperatures for one of the Mazda lamps used, when operated at different voltages as follows:

Voltage volts	Color temperature ° K.
105 .....	3010
110 .....	3060
115 .....	3110
120 .....	3180

lamps used in the spectral regions with which we are concerned; the four curves are for color temperatures corresponding to four different lamp

voltages. The color temperatures at different voltages were obtained from photometric determinations against known standards. For most of our experiments we are not interested in the color temperature so long as it remains reasonably constant throughout a given experiment. The total emission varies widely with the voltage, as will be seen from Figure 2, and in order to control this, the line voltage was stepped up by means of a transformer, and the voltage across the lamp controlled by means of a voltmeter and a carbon disk rheostat. The lamp voltage was not the same for all experiments; in some it was maintained at 120 volts and in others at 115 volts; moreover, the distance from the source was not the same in all experiments, and thus the *threshold times* are not comparable for different experiments but only within a given experiment.

tiveness of the lamp in producing the erythral response when burning at different voltages. Figure 3 shows the results of an experiment to determine this; the logarithms of the *threshold times* are plotted so as to indicate the percentage variation in effectiveness of the incident radiation at different lamp voltages; the shape of the curve has no theoretical significance. An examination of the figures shows that if the voltage is held constant within two volts, the error in the *threshold time* should not be greater than ten per cent. Greater constancy than this was maintained with the exception of occasional slightly greater fluctuations which were of short duration, and probably cancelled out in most cases.

If the lamp is placed close to the skin the full radiation will produce a heat erythema; this appears immediately, and unless severe, fades very quickly. On the other hand, the *urticaria solare* response is always delayed in appearance unless the irradiation is very intense or prolonged, and is much slower in disappearing. Thus there is little danger of confusing the two types of response. In order to eliminate this heat erythema, which in some cases might confuse our results, we have used the water filter described above to remove the infra-red radiation, or in some cases a Corning glass filter (Number 395, extra light shade Aklo, 3.9 mm. in thickness). The effect of temperature on the threshold time will be discussed below.

*The reciprocity law.* The use of the threshold time as a measure of sensitivity is only justified if it can be shown that there is a reciprocal relationship between intensity and duration of irradiation. To test this, the intensity was varied by changing the distance between the light source and the skin, the threshold time being determined for each intensity. Since it was found that the water filter (C, Figure 1) served to focus the light rays and thus prevented the estimation of the light intensity by the inverse square law, it was replaced by the Corning glass filter (Number 395, 3.9 mm. in thickness) which served to remove the infra-red radiation to a sufficient extent to prevent the occurrence of heat erythema. It was also necessary to remove the mirror (E, Figure 1). All the tests were made on the skin of the abdomen, where it had been previously determined that the sensitivity was extremely uniform.

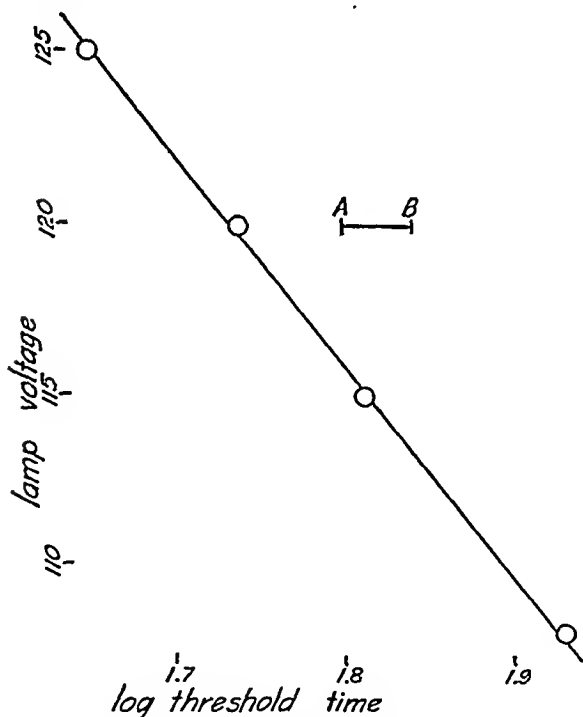


FIG. 3. RELATIONSHIP OF THRESHOLD TIME TO LAMP VOLTAGE.

A-B indicates a variation of 10 per cent in the threshold time.

While it is possible to make some estimate of the variation of emission with voltage from the curve shown in Figure 2, it was thought desirable to make a more direct determination of the effec-

TABLE I  
The reciprocity law

$d$  = distance from lamp in cm.;  $t$  = time in seconds;  
 $I \times t = k = \frac{1}{d^2} \times t$ , where  $I$  = intensity.

Ex-periment	Date	<i>d</i>	<i>t</i>	<i>k</i>	Average	Per cent deviation from average
1	August 29	29.8	95	.103		
		21.9	65	.136		
		15.5	30	.125		
		10.0	12	.120		
					.121±.018	15.0
2	August 31	10.0	14	.140		
		21.6	65	.139		
		50.3	300	.119		
		15.3	30	.128		
		33.2	130	.118		
					.129±.011	8.5
3	September 5	10.0	14	.140		
		50.0	315	.126		
					.133±.700	5.2
4	September 19	15.0	31	.138		
		30.0	100	.111		
		10.0	13	.130		
		50.0	300	.120		
		20.0	55	.137		
					.127±.160	12.5

The results of four experiments on four different days are summarized in Table I. Of these, Experiment 2 was the most carefully conducted and may be taken as a fair index of the accuracy of adherence to the reciprocity law under our experimental conditions. It will be seen that for this experiment, the values for  $k$  in the equation:  $Intensity \times time = k$  vary from the average by about 8.5 per cent, indicating an outside error of less than  $\pm 10$  per cent. The results for the other experiments, which cover a period of three weeks, show average values for  $k$  which agree within  $\pm 5$  per cent, showing that there was little or no fluctuation in the sensitivity over this time; the variations within these experiments are somewhat greater than in Experiment 2. Figure 4 shows graphically the range over which the reciprocity law holds and the deviation of our experimental measurements. It would seem from these data that we may safely assume that the reciprocity law holds for the urticaria response, and that the *threshold time* may be used as a

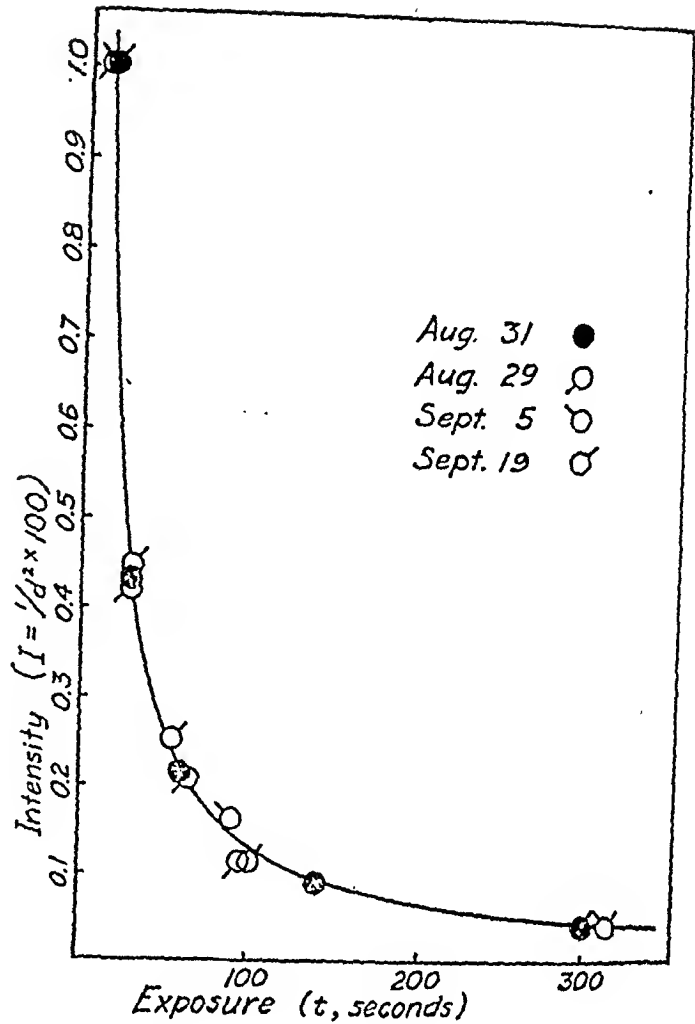


FIG. 4. THE RECIPROCITY LAW.

The curve is drawn from the equation:  $I \times t = 0.129$ .

measure of the photosensitivity with an error of not more than  $\pm 10$  per cent if the conditions are properly controlled.

A point of practical importance to the patient as regards artificial illumination may be mentioned here. From the above data one may calculate that at a distance from the lamp of 3 meters, 3 hours of irradiation would be required to produce a response. Since the lamps ordinarily used for illuminating purposes burn at lower temperatures, their emission is less, and particularly in the shorter wavelengths to which this individual is sensitive. Thus, ordinary conditions of artificial lighting are not bothersome, although even reflected sunlight in a room with light colored walls may be a source of considerable annoyance.

We may make a rough estimate of the quantity of radiation required to produce the response. From the data given by Luckiesch (4) we find

that a black body at  $3200^{\circ}\text{K}$ . emits 6.12 microwatts per square centimeter per foot-candle in the spectral region 4000 to 7600 Å. The color temperature of a tungsten lamp is very close to that of a black body at this range of temperatures (Holladay (5)) so that this value may be taken as sufficiently close for our purposes. From the curve for emission of a black body at this temperature it may be calculated that between one-sixth and one-seventh of the emission between 4000 Å and 7600 Å lies between 4000 Å and 5000 Å, the spectral region which elicits the *urticaria solare* response, so that we may estimate that about one microwatt per sq. cm. per foot-candle is emitted in this region. Our lamp emits about 1300 horizontal foot-candles, and from our data for the reciprocity law we see that at a distance of one foot, the threshold time is about one minute. Thus we may estimate that approximately 1300 microwatts of radiant energy of wavelengths 4000 to 5000 Å must fall on the skin of this individual in order to produce an urticarial response in one minute.

*The effect of temperature on the response.* It was recognized early in the investigation that temperature might have a considerable influence on the response under certain circumstances. Studies were therefore attempted to determine the magnitude of this effect so that an estimate of the influence of temperature on the accuracy of our measurements might be made.

The effect of temperature on the *threshold time* was first determined. For these experiments the stop *B* was replaced by a thin black paper stop with a 2 cm. hole, placed tightly against the water filter *C*, no color filters (*D*) being used. The skin of the abdomen was held firmly against this black paper so that the skin area next to the water filter would tend to take the temperature of the water which could be varied by passing different mixtures of hot and cold tap water through the filter. The temperature of the filter was determined by means of a thermometer placed in the outlet stream from the filter, and the surface temperature of the skin corresponding to any given filter temperature was estimated as follows. The skin was held against the filter for a given period of time, then moved away, the time noted, and the surface skin temperature determined as

quickly as possible by means of a thermopile designed for this purpose, the time at which the measurement was made being carefully noted. Further measurements were made at successive intervals as the skin temperature changed toward the normal, and from these successive measurements a curve was plotted and an extrapolation made to zero time which should correspond to the temperature of the skin when in contact with the filter. It was found that for periods shorter than five minutes in contact with the water filter the values obtained for skin temperature varied considerably, but that when the skin was allowed to remain in contact for periods as long as twenty minutes, the values were very little different than those obtained for the five-minute periods. In our subsequent experiments we therefore kept the skin in contact with the filter for five minutes before the beginning of each experiment, i.e., before beginning the irradiation of the skin. From data obtained for the skin surface temperature at various filter temperatures we were able to plot the curve shown in Figure 5, from which an estimate of the skin surface temperature could be made by merely measuring the temperature of the water at the filter outlet. It is doubtful if these estimates for the skin surface temperatures can be regarded as within better than one or two degrees of the actual skin surface temperature, and the temperature inside the skin where the response takes place must have been somewhat different than those of the skin surface. It would seem that the range of temperatures inside the skin would be somewhat less than those on the surface, but we have no way of estimating this difference.

Measurement of the *threshold time* at different temperatures was made as follows. The skin was held against the filter for a period of five minutes in order to establish the temperature of the skin. The lamp was then turned on for a given irradiation period, the skin being maintained in contact with the filter during this time. The skin was then moved away, and allowed to adjust toward normal temperature while observation for the appearance of a threshold erythematous response was made. By repeating this procedure for different periods of irradiation, the *threshold times* for the response at given temperatures were determined. Data for a series of such measurements are plotted



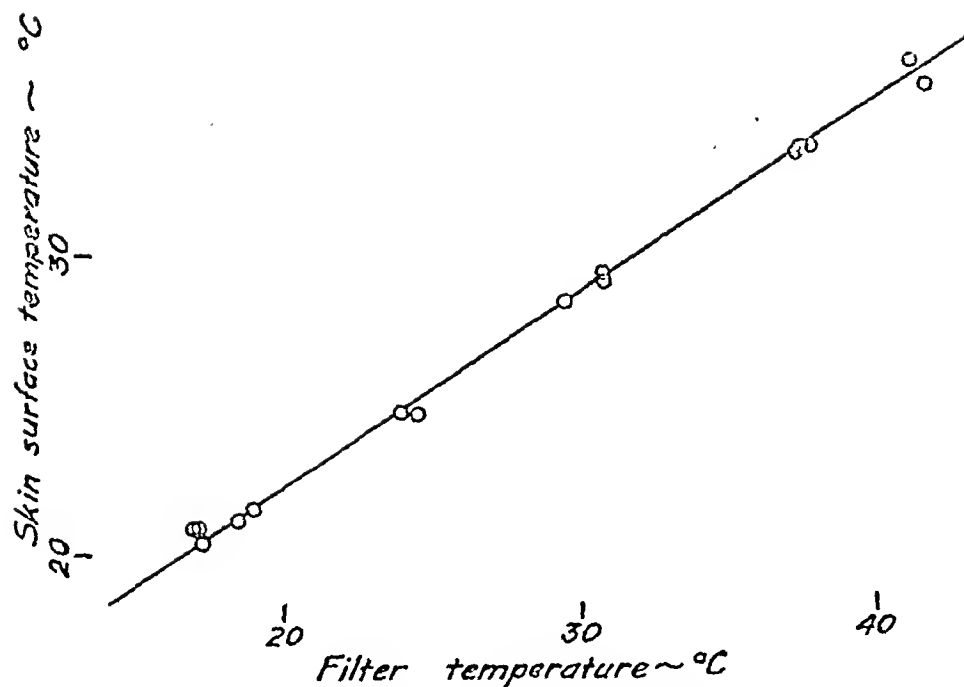


FIG. 5. RELATIONSHIP OF FILTER TEMPERATURE TO SKIN TEMPERATURE.

in Figure 6. From these data it appears that the  $Q_{10}$  for the *threshold time* is about 1.3 to 1.4 over the range studied. This is a quite reasonable coefficient for a photochemical reaction.

Reference to Figure 6 will give some indication of how differences in temperature may affect the experimental determination of *threshold time* in

the rest of our experiments. We may assume that the normal temperature of the skin surface of the abdomen when exposed to ordinary room temperatures is about 30° C., and in this region we see that a change of about 3° C. would result in a variation of 10 per cent in our measurements of *threshold time*. This is a much greater tem-

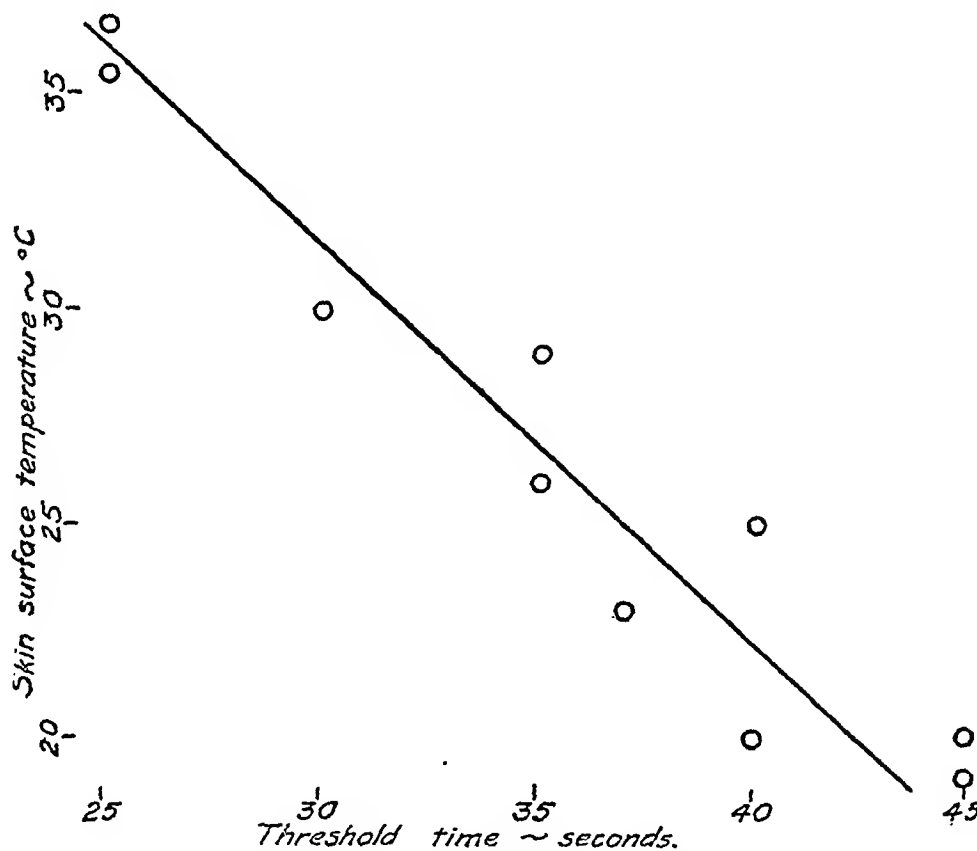


FIG. 6. EFFECT OF TEMPERATURE ON THRESHOLD TIME.

perature variation than is to be expected if the room temperature is maintained reasonably constant as was the case in our experiments, but we see that temperature variation is a factor which cannot be entirely neglected, particularly in comparing experiments made on different days.

The low temperature coefficient of the *threshold time* would indicate its rather direct relationship to the primary photochemical mechanism. However, the rate of development of the urticarial response as indexed by its latent period is not taken into account in the measurement of *threshold time*.

using the same period of irradiation, but a shorter total period in contact with the filter, or if the erythema is not present, the total period is increased. By a series of such trials the least time required for a minimal erythema to appear is determined and may be taken as an index of the rate of development of the erythema response for the given temperature and period of irradiation. By repeated experiments using different temperatures and different periods of irradiation a series of such measurements were obtained which are displayed in Figure 7. Since the primary photo-

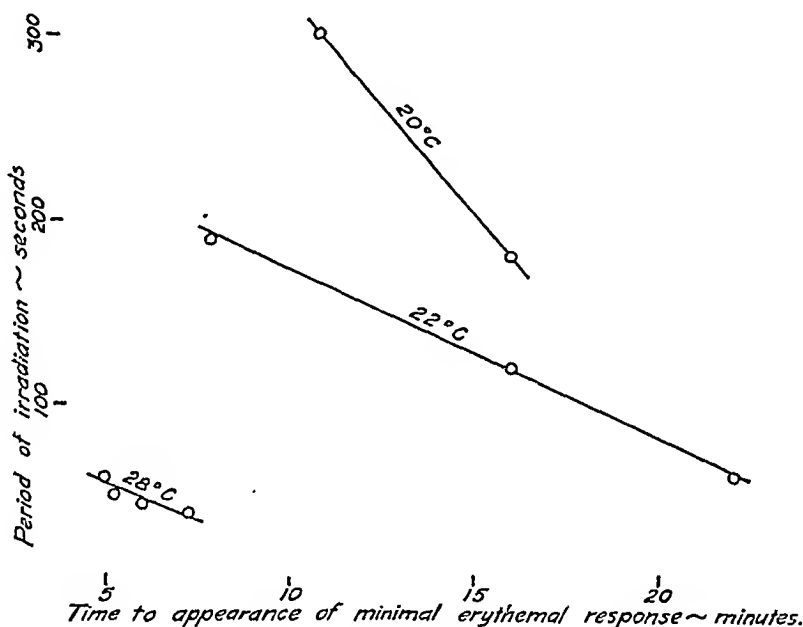


FIG. 7. EFFECT OF TEMPERATURE ON LATENT PERIOD.

The latent period is shortened as the period of irradiation is decreased. We have attempted to measure the effect of temperature on the rate of development of the erythema reaction after the end of the irradiation period, as follows. The skin is held against the water filter for five minutes, the lamp then turned on, and irradiation continued for a given length of time which is known from the data of Figure 6 to be longer than the *threshold time* for the particular temperature. When the lamp is turned off, the skin is kept in contact with the filter for a further period of time, and is then moved away and observed for the minimal erythema response. If the erythema is observed to be present at the time the skin is moved away from the filter, the experiment is repeated

chemical reaction has a low temperature coefficient, the period of irradiation may be taken to represent the production of the same quantity of reactants at all temperatures used, and the rates measured for different temperatures when the same period of irradiation was used should be subject to comparison. Unfortunately, these measurements are laborious and trying to the subject, and the measurements which we have made are scanty and rough for this reason; they seem, however, to be significant.

The data collected in Figure 7 show that the time of appearance of the threshold erythema response is greatly lengthened at low temperatures. In fact, the effect is so great that it is difficult to obtain comparable data over a wide range of

temperatures, and no attempt has been made to calculate the temperature characteristics of this response. Furthermore, when a temperature of 35° C. was maintained in the filter and the skin kept in contact with it for a long enough period of time (over seven minutes), no response appeared, so that the temperature coefficient could not be determined in this region. Our results would seem to be quite comparable to those obtained by Lewis (2, Chapter VII), who found that either low (12 to 15° C.) or high (45 to 47° C.) temperatures inhibit the appearance of the triple response following histamine pricks in normal individuals, or stroking in urticarial subjects. Lewis has explained this as due, in part at least, to changes in the local circulation produced by changes in temperature, and if we invoke the same explanation it would seem meaningless to attempt to determine the temperature characteristics accurately, since they would not be an index to the specific reactions of *urticaria solare*, but of more general reactions.

Lewis has suggested that the triple response in other types of urticaria results from the release of a histamine-like *H* substance from the cells of the skin, and it seems reasonable to extend this concept to *urticaria solare*. The similarity of behavior with respect to temperature is added evidence to justify this position. We might, then, suggest the following scheme to represent the mechanism of *urticaria solare*:

1.  $S + h\nu \rightarrow S_r$
2.  $S_r + \text{cells} \rightarrow H$
3.  $H + \text{vessels} \rightarrow \text{triple response}$

In the primary reaction, 1, *S* is the light absorbing molecule in the skin which is responsible for the initiation of the response,  $h\nu$  is a quantum of light absorbed by *S* ( $h = \text{Planck's constant}$  and  $\nu$  the frequency of the radiation), and *S<sub>r</sub>* the reactive molecule resulting. By *S<sub>r</sub>* we do not wish to imply an activated molecule in the strict photochemical sense, but merely to indicate that the molecule *S* has been in some way modified and enabled to take part in a subsequent reaction. In reaction 2, *S<sub>r</sub>* reacts with skin cells to release the histamine-like substance *H*, which then reacts with the small blood vessels to produce the triple response 3. Obviously reaction 2 may be a chain reaction involving many steps. Reaction 1 must

have a very low temperature coefficient since it is a purely photochemical reaction, and its rate is not dependent on the energy of activation but on the capture of light quanta. However, reactions 2 and 3 are thermal reactions and may have high temperature coefficients. It seems probable that reaction 3 is the one which dominates the picture when the effect of temperature on the rate of appearance of the *urticaria solare* response is studied, because of the similarity of behavior to that obtained when histamine is introduced directly into the skin. The temperature coefficient obtained for the *threshold time* is probably determined principally by reaction 1 which would account for its low value; reaction 3 probably plays little part in the determination of the *threshold time* where the temperature is only maintained during the period of irradiation, and the development of the response takes place at approximately normal skin temperature.

TABLE II

*Sensitivity on various regions of the body, December 3, 1935*

Region	Threshold time
Ventral surface of abdomen..	60 seconds
Lumbar region of back.....	60 seconds
Back over scapula.....	70 seconds
Medial surface of thigh near knee.....	165 seconds
Outer surface of forearm (15 cm. above wrist).....	150 seconds
Inner surface of forearm (15 cm. above wrist).....	105 seconds
Inner surface of forearm at wrist.....	180 seconds
Palm of hand.....	180 seconds (itching only, no observable erythema)
Dorsum of hand.....	Longer than 20 minutes
Cheek.....	Longer than 20 minutes

*Topographical distribution of photosensitivity.* Determinations of the *threshold time* for a number of regions of the body are given in Table II. A constant light intensity was maintained throughout, and all the determinations were made within a period of three hours. The sensitivity varies widely over the body, the abdomen and lumbar region of the back being at least twenty times as sensitive as the face and the back of the hands. The surface of the abdomen is uniformly sensitive within the limits of our experimental error, and we have used this region in all our other experiments. It is of interest to note how the sensitivity may vary in adjacent regions, *e.g.*, compare

the palm and dorsum of the hand. Although we were unable to produce any erythemic response by exposure of the face and dorsum of the hands to our light source for twenty minutes, it must not be judged that these parts have become entirely insensitive, for the response can still be elicited by exposure to sunlight, and the subject is still made uncomfortable by such exposure, although to a much less extent than two years ago. Unfortunately, no measurements of this kind were made early in the development of the disease, the figures in Table II being obtained about nine months after its onset. We cannot, therefore, make a definite statement that the decreased sensitivity of the exposed parts has developed as a result of exposure to light; but judging from the subject's reaction to casual exposure to sunlight at the onset and at present we feel no doubt whatsoever that this is true. Moreover, Duke (6), Valery-Radot (7), and Blum, Allington and West (1) have all found that exposure to light has some effect in decreasing the sensitivity of the skin to light.

Numerous possibilities offer themselves for the explanation of the difference in sensitivity of various regions of the skin, and the mechanism of desensitization of local areas. Among these arises the question of the thickness of the skin, and its relative transparency to light. Miescher (8) has pointed out that the thickness of the skin is very important in determining the sensitivity of normal skin to the erythema producing ultraviolet radiation (principally shorter than 3200 Å), and that the decrease in sensitivity to such radiation after exposure to it, may be due to the thickening of the epidermal layers. The data of Bachem and Reed (9) for the absorption of different wavelengths of light by the various layers of the skin, afford the opportunity to make some estimate of the effect of the thickness of the epidermis on the normal erythemic response and on the *urticaria solare* response. The normal erythemic mechanism would seem to be set off in the epidermis, probably chiefly in the malpighian layer, since there is very little penetration (9 to 16 per cent) of the exciting wavelengths below this layer, since the malpighian layer is the principal site of pigment deposition which follows the erythema (Laurens (10)), and since the erythema is delayed as though time might

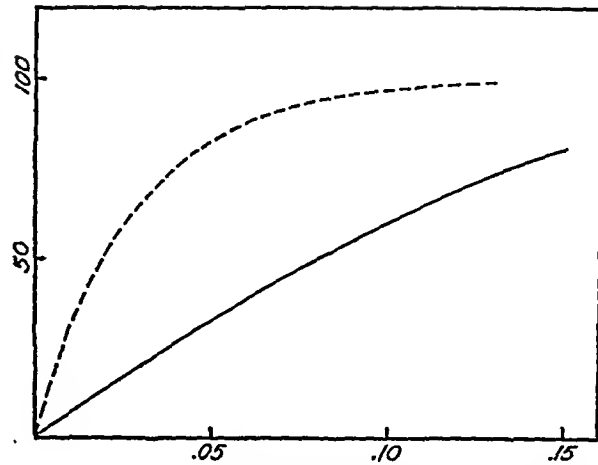


FIG. 8. EFFECT OF THICKENING OF SKIN ON PENETRATION OF RADIATION.

Abscissa—thickening in mm. Ordinates—per cent reduction in radiation reaching the photosensitive layer. Broken line—3000 Å, malpighian layer (normal erythemic response). Solid line—4500 Å, papillary layer (*urticaria solare*).

be required for the products resulting from the irradiation to reach the papillary layer where the first blood vessels are found. In Figure 8 has been plotted the percentage reduction in radiation of wavelength 3000 Å reaching the malpighian layer which would be caused by thickening of the corneum, based on the data of Bachem and Reed which is for skin from the region of the flexor surface of the arm and the abdomen. It will be seen that a thickening of 0.03 mm., which amounts to doubling the thickness of the corneum, would reduce the radiation reaching the malpighian layer by about 66 per cent, so that an amount of thickening which would be difficult to observe would cause a considerable difference in the sensitivity of the skin to radiation of this wavelength. Thus small differences in the thickness of the skin at different regions of the body would produce marked variations in sensitivity, and it is conceivable that thickening resulting from irritation caused by irradiation would account for at least part of the resistance of the skin to ultraviolet radiation subsequent to exposure (Laurens (10)).

The case of the *urticaria solare* response is somewhat different, the radiation which evokes it being transmitted by the skin to a much greater extent. The response appears almost immediately after the irradiation, in contrast to the response

elicited by ultraviolet light, which suggests that the locus of action is close to the small vessels which first appear in the papillary layer. Upon reference to the data of Bachem and Reed (9), we find that only about 20 per cent of the total light of wavelengths 4000 to 5000 Å incident upon the skin is absorbed in the epidermal layers, but that 50 per cent is absorbed in the papillary layer, so that it is quite possible that the *urticaria solare* response is elicited in the latter layer. In Figure 8 is plotted the percentage decrease in radiation of wavelength 4500 Å, which results from increasing the thickness of the epidermis; this indicates that the thickness of the epidermis should have much less effect on the *urticaria solare* response than on the erythemic response to ultraviolet radiation. From Figure 8 it may be seen that a thickening of 0.1 mm. which amounts to tripling the thickness of the epidermis would be required to reduce the light reaching the papillary by 60 per cent. While differences in the thickness of the skin might be an important factor in determining the *threshold time* for the various regions of the body, it is improbable that it is a very important factor in the desensitization of the skin.

Blum, Allington and West (1) found that successive irradiation of an area of the skin with the quartz-mercury arc in quantity sufficient to produce a strong pigmentation reduced the sensitivity of that area to a marked degree. A filter was interposed (Corning 986) to remove the radiation above 4000 Å, so that the *urticaria solare* response was not elicited during the building up of the pigment. As we have pointed out above, thickening of the epidermis could hardly account for a large part of the decrease in sensitivity. The pigment, which is deposited in the basal cells of the malpighian layer of the epidermis, may be very effective as a filter if the *urticaria solare* response originates in the papillary layer provided it absorbs strongly in the blue-violet region, and may conceivably be the cause of the apparent desensitization in the experiment of Blum, Allington and West. However, at the time the tests recorded in Table II were made, the face of the subject showed very little pigmentation so that the lack of sensitivity of this region could not be credited to any extent to this factor.

Another possibility is that the exposed areas of

skin in which the urticarial response has been frequently produced by the action of light, has decreased in sensitivity to the products of the photochemical reaction; for instance, its sensitivity to histamine. This was tested by pricking histamine into various regions of the skin, and comparing the reaction with that of a series of normal individuals.<sup>2</sup> The subject's reactions to histamine were within normal limits on all parts of the body including the exposed parts. The sensitivity to histamine is normally less on the exposed parts, but the difference is not of the order of the difference in sensitivity to blue-violet light shown by our subject. Reactions to adrenalin pricks were also within normal limits. Thus we have no reason to suspect an abnormal vascular reactivity in the subject.

Finally, we come to the concept that exposure to light exhausts some part of the photochemical mechanism. This is the suggestion of Duke (11). We must admit this as a distinct and reasonable possibility, but one that cannot be categorically accepted in face of the other possibilities cited above, unless further proof can be found. The fact that Blum, Allington and West (1) were able to produce a decrease in sensitivity by ultraviolet irradiation without eliciting the *urticaria solare* response demonstrates that some other factor is involved than the wearing out of the photochemical mechanism. It is probable that all the factors mentioned play a part in decreasing the sensitivity of the skin which is exposed intermittently to sunlight.

*Variations in the general sensitivity with time.* An attempt was made to follow the sensitivity of a region of the skin normally covered by clothing, the abdomen, over the course of ten months. These determinations were subject to a greater error than our other studies, because it was impossible to use the same lamp throughout the entire series, and because in some cases the voltage was not carefully controlled. Moreover, at the time of the earlier measurements we were not aware of some of the possible errors in the detection of the threshold. In general, the later determinations are more trustworthy, but it is difficult

<sup>2</sup> These tests were made through the courtesy of Dr. Eric Ogden who will publish an account of the method and results of a series of such tests at a subsequent time.

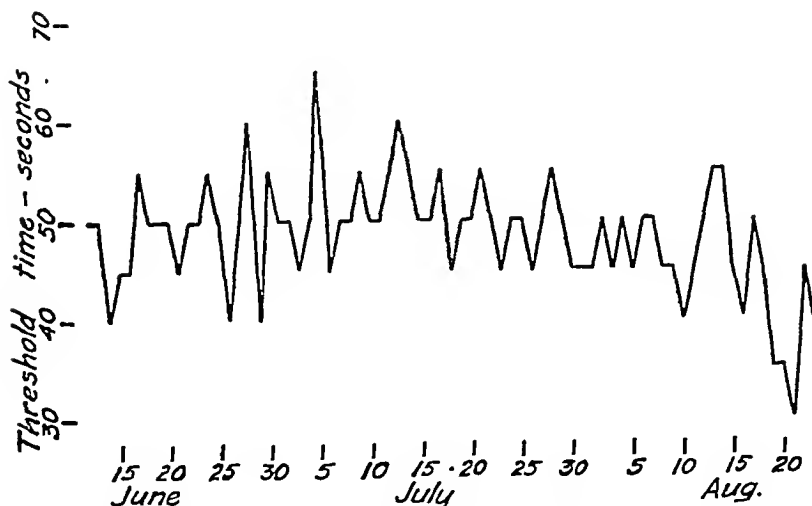


FIG. 9. VARIATION OF THRESHOLD TIME DURING A PERIOD OF TWO MONTHS.

to see how the error could be greater than 50 per cent throughout the series. Figure 9 shows a series of measurements carried over a period of three months which are probably comparable. It shows no general trend of sensitivity over the whole period of three months, but demonstrates that there are fluctuations of short duration which are undoubtedly greater than the estimated error. These results indicate that it is probably important to perform a given experiment on a single day, and this has been our policy. They also indicate that in any attempt at therapy one should not be misled by minor changes in sensitivity which may be only ephemeral.

The relative stability of the photosensitivity indicates that the photochemically active substance, whatever its nature, is constantly renewed. It may be a product of abnormal metabolism, a substance elaborated by a parasite, or a substance regularly introduced in the diet. In consideration of the latter possibility, some attempt was made to eliminate various factors from the diet, but without any observable effect on the photosensitivity.

*Miscellaneous skin tests.* As has been indicated in the preceding discussion, there is no reason for believing that an allergic reaction in the usual sense of the word is involved in the *urticaria solare* response. The only assumptions which are necessary to explain the response are that a photoactive substance is abnormally present which, when activated by light, initiates reactions which result in the production of a histamine-like

substance. Nothing is definitely known about the intermediate steps except that they are not of the "photodynamic" type since they do not require molecular oxygen as was shown by Blum, Allington and West (1) and Blum, Watrous and West (12). There seems no reason for assuming that they are related to allergic reactions, but we have searched for an allergic background since it is commonly suspected in urticarial cases. No history of allergy either in the patient or members of his family was obtained, and scratch tests with a large number of food, pollen, and epidermal extracts gave only negative results. Reactions to heat and cold and to stroking are normal, and there is no dermatographism. Thus no evidence of an allergic background for this condition has been found.

Passive transfer of the sensitivity was attempted by intradermal injection of serum from the patient into normal skins. In preparing the serum, the active blue-violet light was excluded, in order that any photolabile substance might not be destroyed. No uniform differences were found between the reactions to this serum and those to serum from a normal individual. The serum was then exposed to light and the injections repeated, but with similar results. Exposure of the injected area to light also failed to provoke any abnormal response.

Prompted by the close association of a bcc sting with the onset of the photosensitivity we tested the patient's sensitivity to an extract of bcc

venom, prepared as suggested by Thompson (13) by triturating the poison apparatus in normal saline and filtering through a Seitz filter. One-tenth cubic centimeter of a solution estimated to contain 1 to 1500 parts of venom when injected intradermally provoked a wheal approximately one centimeter in diameter showing free pseudopodia. In normal individuals the usual result is an erythematous papule less than half this size, so that the patient seems somewhat sensitive to bee venom. Suspecting some relationship between this sensitivity to bee venom and to light, attempts were made to desensitize the patient by a series of injections of the dilute bee venom solution, but without any definite effect on the sensitivity of the patient to light. Simultaneously and subsequently the patient was given hydrochloric acid by mouth to correct a condition of achlorhydria, but no change in sensitivity to light resulted.

*The active wavelengths.* As stated above, the preliminary studies by Blum, Allington and West (1) delimited the wavelengths which produce this urticarial response to the region between 3900 and 5300 Å, which is in very good agreement with the findings of Duke (6), Vallery-Radot (7), and Frei (14) for their cases. We must assume that whatever the ultimate chemical reaction which produces this urticarial response, the primary reaction is the absorption of a quantum of light by a molecule of some photoactive substance in the skin as represented in reaction 1 above, and whatever this substance may be, it must have a characteristic absorption spectrum, i.e., there must be only restricted regions of the spectrum which it can absorb, and consequently only light of these spectral regions can bring about the urticarial response. We dwell upon this fundamental point because it has been too frequently disregarded in the study of photosensitivity in man. The relative sensitivity to different wavelengths should correspond to a certain degree with the amount of light absorption of the photosensitizing substance, and the curve relating sensitivity and wavelengths should approximate the absorption spectrum of that substance. There are, of course, a number of factors which would tend to produce some disagreement in these curves, such as the effect of the solvent on the absorption spectrum of

the photoactive substance, the specific absorption of the skin, variations in photochemical efficiency with wavelength, etc.; but if we are able to obtain a sensitivity wavelength curve for the photo-dermal response, we have a basis for a guess as to the nature of the photosensitizing substance. For this reason we have attempted to make some more quantitative determinations than those previously reported.

In these studies colored glass filters were used to isolate relatively narrow bands of wavelengths. The transmissions of the various filter combinations were determined for the visible region of the spectrum by means of a spectrophotometer. Since we had already determined by means of glass color filters (Blum, Allington and West (1)) and sunlight or quartz mercury arc that wavelengths outside the 3900 to 5300 Å region were ineffective, we could disregard any filter transmission outside of this restricted region. The spectrophotometric measurements could not be made for wavelengths shorter than 4300 Å, and it was necessary to extrapolate to zero transmission beyond this point, but our preliminary determinations would indicate that the sensitivity below this wavelength is so slight that no significant error could have been introduced.

The lamp used in these particular experiments was the one whose emission curves are plotted in Figure 2; it was operated at 120 volts, at which voltage the color temperature was 3180° K. The emission curve was calculated for a black body at this temperature by means of Wien's equation (see Harrison (3)), and the transmission of each filter combination for given wavelengths was multiplied by the emission for the corresponding wavelengths; thus curves were obtained for the relative quantities of radiant energy incident through the various filters. A further correction was made to give a relative number of quanta by multiplying by the wavelength of the radiation.<sup>3</sup>

<sup>3</sup> Since the amount of photochemical reaction depends upon the number of quanta absorbed by the photochemically active substance  $S$ , it is proper to compare the relative number of quanta rather than the relative energies corresponding to the exciting wavelengths. The correction is so small in this case as to be of little significance, however. The subject is discussed by Blum and Scott (15), in which paper Equation 8 should read:  $N\lambda' = I_{\lambda} S_{\lambda} T_{\lambda} (-\log T_{\lambda})$ .

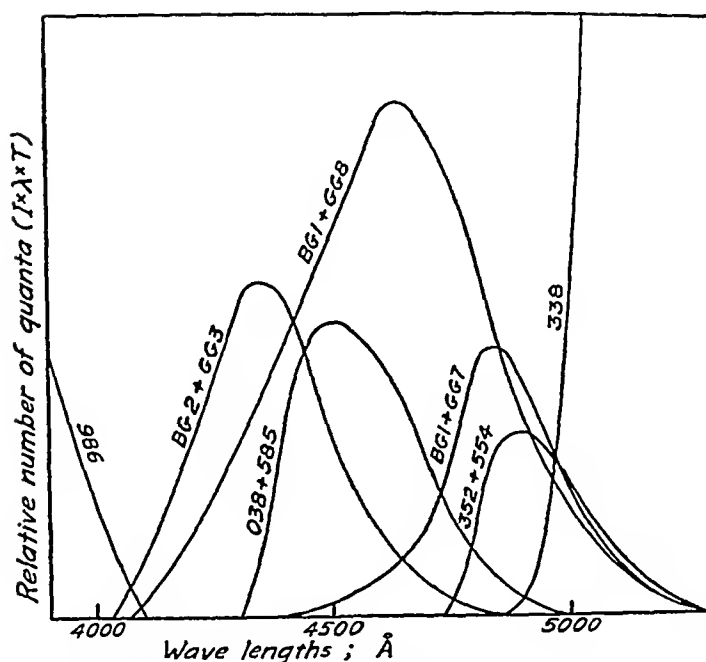


FIG. 10. LIGHT PASSING THROUGH FILTERS USED TO ISOLATE SPECTRAL REGIONS.

The curves for the relative number of quanta passing through the filter are shown in Figure 10.

TABLE III

*Photosensitivity to restricted spectral regions*

(Experiments 1, 2, and 3 were performed on October 12, October 29, and October 30, respectively)

Filter	$\lambda$ Maximum Å	A	Ex- peri- ment	t seconds	$k_\lambda = A \times t$	$\frac{1}{k_\lambda}$
BG2 + GG3	4350	.44	1	150	66	.015
			2	150	66	.015
			3	195	86	.012
						Average = .014
038 + 585	4500	.38	1	90	42	.025
			2	90	42	.025
			3	85	39	.036
						Average = .025
BG1 + GG8	4650	1.00	1	90	90	.011
			2	70	70	.014
			3	70	70	.014
						Average = .013
BG1 + GG7	4850	.32	1	165	53	.019
			2	195	62	.016
			3	165	53	.019
						Average = .018
352 + 554	4900	.19	1	330	64	.016
			3	300	58	.017
						Average = .016
986				300 (no response)		
338				100 (heat response)		

The relative number of quanta reaching the skin through each filter should be proportional to the area under the curves. These areas, measured by means of a planimeter, are expressed in relative units as  $A$  in Table III.

The threshold time for the erythral response was determined for each filter, the values for three experiments being given in Table III. Since the reciprocity law holds for light made up of all wavelengths, it may be assumed that it also holds for the restricted spectral bands which pass through the filters. Thus the product of  $A$ , which is a measure of the relative number of quanta passing a given filter, and the threshold time  $t$ , should give a value  $k_\lambda$ , which is a measure of the relative number of quanta required to elicit a response at that wavelength. The reciprocal of  $k_\lambda$  should be a measure of the relative sensitivity at this wavelength, and is the value which may be compared with the absorption of a substance suspected of being the photochemically active agent. Assuming the values of  $1/k_\lambda$  to represent the sensitivity of the *urticaria solare* response at the wavelengths corresponding to the maximum transmissions of the various filters, it would appear that the sensitivity has two wavelength maxima, one at about 4500 Å, and another at about 4900 Å,



with a distinct minimum at about 4650 Å. That there is little sensitivity above 5000 Å or below 4000 Å was shown clearly by the earlier experiments of Blum, Allington and West (1). Obviously, these values of  $1/k_\lambda$  are subject to a considerable degree of error in both parameters, in the one case due to the experimental error involved in determining the threshold, and in the other due to the fact that the band of light transmitted by the filter is rather wide; the degree of error in the latter case cannot be accurately estimated. Another error is introduced, of course, in the determination of the color temperature of the lamp which might somewhat alter the relative values, although this could hardly affect the position of the maxima. At best, these values cannot be considered as very accurate, but they might be expected to give an approximate picture of the absorption spectrum of the photosensitizing substance which is responsible for the urticarial response.

We may now begin our search for the absorption spectrum of some biological substance which will fit this region of absorption with a reasonable degree of approximation. By reference to the data on absorption bands accumulated in *Tabulae Biologicae* (16, 17, 18, 19, 20) we find that only one group of pigments there listed has the maxima of absorption of its members confined to the general spectral region which elicits the *urticaria solare* response; these are the carotenoids. As pointed out by Blum, Allington and West (1), the porphyrins, which are active photosensitizers, exhibit a minimum of absorption between 4000 and 5000 Å (21); the bile pigments show no maxima which will correspond with the region of sensitivity as is also true for cytochrome and hemochromogens in general (22). The flavines, another group of naturally occurring photolabile pigments, absorb in the same general spectral region as the carotenoids (4000 to 5000 Å), but show greater absorption in the near ultraviolet (23); so that if one of these were the sensitizer in the present instance, we should expect to find photosensitivity in the corresponding spectral region where the individual with *urticaria solare* exhibits little or no sensitivity.

Thus the carotenoids which are definitely photolabile seem to offer the best agreement with our

experimental data, and in Figure 11 we have plotted the absorption spectrum of  $\alpha$  carotene in alco-

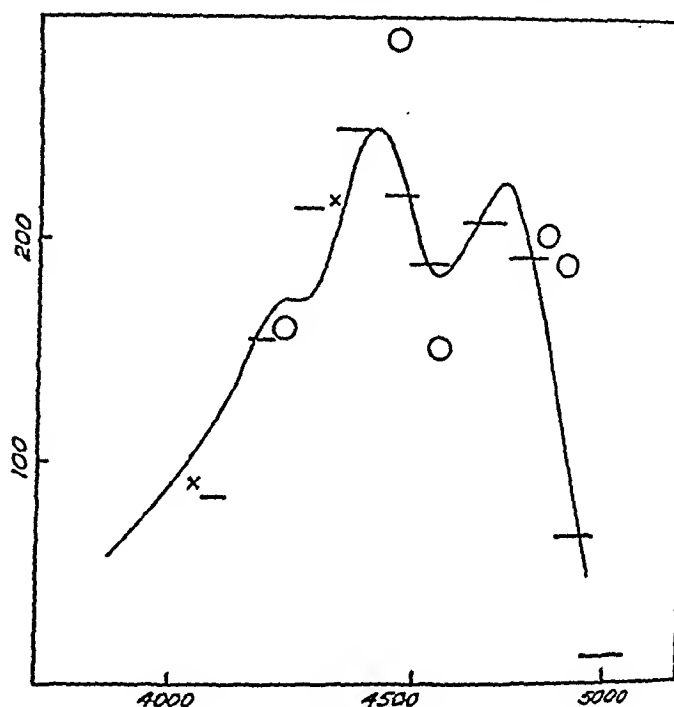


FIG. 11.

Abscissa—Ångstrom units; ordinates—arbitrary values. Circles—relative sensitivity of *urticaria solare* ( $1/k$ ). Horizontal lines and crosses—relative sensitivity of phototropic bending of the oat seedling. From Johnston (25), corrected to relative number of quanta. Curve—absorption of  $\alpha$  carotene in alcohol. From Miller, Mackinney and Zscheile (24).

hol (24), together with our experimentally obtained values  $1/k_\lambda$  for the *urticaria solare* response. In the figure the ordinates for both were chosen so as to bring the absorption curve of  $\alpha$  carotene and the values of  $1/k_\lambda$  into relationship. It will be seen that at least a rough agreement exists.

In Figure 11 we have also plotted spectral sensitivity data for the phototropic response of the oat seedling, as given by Johnston (25), choosing our ordinates so as to bring the data into accordance with the absorption curve of  $\alpha$  carotene. The agreement is rather good, but we could not from this make a categorical statement that  $\alpha$  carotene or any other specific carotenoid is the photoactive substance responsible for phototropism in the oat seedling, although we must admit that possibility which has been previously suggested by Bachmann and Bergann (26). We note the existence of two distinct maxima in the data of Johnston corresponding approximately to the

two maxima in our own data for the *urticaria solare* response. Other data for the phototropic bending of the oat seedling do not agree quantitatively with that of Jolinston (see Bachmann and Bergann (26); Haig (27)), but all the measurements agree in the delimitation of the general spectral region and in general in the display of two maxima. In considering the data from several sets of measurements on the phototropic bending of the same organism, the oat seedling (*Avena sativa*), we see that the deviations among the various sets of measurements are as great as the deviation of our own measurements from any one of the above, even though our own data are admittedly of a relatively low degree of accuracy. Thus, so far as the evidence goes the photochemically active substance may be the same in both. Carotenoid pigments have been suspected as the photosensitive materials in phototropic bending of plants, and Castle (28) has been able to extract such a pigment from phycomyces whose absorption spectrum fits well with the spectral sensitivity curve which he has determined for that organism, and which would also approximate our own data and that for the oat seedling. This discussion of the spectral sensitivity of the orienting mechanism in plants has been introduced principally to show the deviation in measurements obtained on other living systems. Numerous factors exist which would create differences between the curves of spectral sensitivity of the living organism, and the absorption curve of the responsible photochemically active substance when removed from the living tissue and in solution in some solvent other than that in which it is dissolved in the organism. For example, the difference in transmissivity of the epidermis to the various active wavelengths might alter the effectiveness of the various spectral bands in eliciting the *urticaria solare* response; but from the data of Bachem and Reed (9), we may estimate a rather uniform decrease in transmittency of about 15 per cent from 5000 to 4000 Å, which could not greatly alter our sensitivity curve. Again, it is unfair to make a comparison of the absorption spectrum of a carotenoid pigment in solution in alcohol with the spectral sensitivity curve of an organism in which the absorbing pigment must be in solution in some other solvent. The ab-

sorption spectra of the carotenoids are shifted very markedly with the solvent employed, so that it is impossible to select any specific carotenoid as showing better agreement with the data than another. The choice of  $\alpha$  carotene in alcohol for plotting in Figure 11 was made only because it showed the possibility of agreement, not to indicate that this is the actual carotenoid involved. An examination of the absorption spectra in the monograph of Zechmeister (29) will show the extent of the shift of the absorption spectra of carotenoids with the solvent, and will also show that these spectra display two principal maxima separated by a well defined minimum, no matter what the solvent. The apparent existence of two maxima in our measurements of the spectral sensitivity of *urticaria solare*, and of the phototropism of *Avena* gives strong support to the hypothesis that carotenoid pigments are responsible for both photo-physiological responses.

Until other evidence is offered, then, we must suspect that a carotenoid pigment is the photosensitizing agent in the case of *urticaria solare* now in hand, and may use this as a working hypothesis. Following this evidence we have attempted to produce local sensitivity to light by injecting solutions of carotene and xanthophyll into rabbits' ears and into human skin, but exposures of the injected areas to sunlight for periods as long as twenty minutes produced no response which could be taken as definite evidence of photosensitivity. The solvents used for injection were cottonseed oil and propylene glycol. In both cases, particularly the former, a considerable irritation was produced by the injected material which may have masked any response resulting from exposure to light.

#### DISCUSSION

The evidence presented in the preceding pages would best be explained by the postulation that a carotenoid pigment is present in the skin, which may be activated by light to set off a series of reactions which result in the release of *H* substance in the region of the small blood vessels. The ultimate result is the appearance of the triple response on the area of skin reached by the light. The only abnormal part in such a mechanism is the presence of the carotenoid pigment, and it will be well, therefore, to inquire into the plausi-

bility of the presence of such a pigment, and its possible origin. Carotenoids are taken up in large quantities in the normal diet, a certain fraction being changed into vitamin A, and a large part excreted in the feces. In some cases, after ingestion of great quantities of food rich in carotenoids, enough may accumulate in the skin to give the individual a yellow or reddish color (29). Hess and Myers (30) found that infants fed an excess of carrots assumed a yellow coloration, and that carotene could be isolated from the blood and urine in such cases; there seems to be no record of sensitivity to light, but Klose (31) states that the yellow color is most pronounced on the parts normally exposed to light.

It would seem thus that great quantities of carotenoid pigments may be present in the skin without sensitivity to light, at least in a degree comparable with that of our subject. Moreover, our patient does not show a general yellow tint, so that there can be no great excess of carotenoids. It seems probable that any carotenoid entering the skin from the blood stream would be deposited principally in the fat of the subcutaneous layers because the carotenoids are very soluble in fat and insoluble in water, and the observations of Klose (31) would indicate that this is the point of deposition. The penetration of blue and violet light to the subcutaneous layers is not great, and for reasons discussed above it seems probable that the *urticaria solare* response is set off in the papillary layer of the corium. It would seem necessary to assume from this that in our subject the carotenoid is deposited superficially to the subcutaneous fat, that it is not present in great quantities, and that it owes its effectiveness as a photosensitizer to its position in the skin or that it is a specific kind of carotenoid which is not ordinarily present in the human organism. All this suggests that the carotenoid may be produced in the skin through the agency of a parasite. It is therefore worthy of remark that our subject displays a few of the yellow macules of *Tenia versicolor* which result from infection of the skin by the fungus *Malassezia furfur*, and that it may be this organism which is producing the photosensitizing carotenoid. We have been unable, however, to show that the yellow macular areas are any more sensitive than parts of the skin which appear

free from the infection, so that this hypothesis receives no substantiation for the present.

#### SUMMARY

Photo-physiological studies of an urticarial response elicited by blue and violet light demonstrate the following.

1. The response obeys the reciprocity law.
2. Studies of the effect of temperature indicate that the mechanism of the response includes a photochemical reaction which is not greatly affected by temperature, and a thermal reaction which is greatly modified by changes in temperature. The latter is probably the action of a histamine-like substance on the small vessels of the skin.
3. All parts of the body are sensitive to light but the degree of sensitivity varies from region to region. The exposed parts are much less sensitive than the parts covered by clothing. The reasons for these variations are discussed.
4. The sensitivity fluctuated somewhat with time, but there was no general trend in the course of ten months.
5. Determination of the spectral sensitivity suggests that the photosensitizer is a carotenoid pigment. The possible origin of such a carotenoid in the skin is discussed.

We wish to express our appreciation of the assistance graciously given by the following: Dr. H. V. Allington who made the clinical studies; Professor L. M. K. Boelter who determined color temperatures of lamps; Professor Eric Ogden for histamine tests; Professor A. P. Krueger for bacteriological control of materials for injection; and Doctors G. Mackinney and S. Lepkovsky who provided samples of xanthophyll and carotene.

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# VARIATIONS IN THE PERMEABILITY OF THE RED BLOOD CELLS IN MAN, WITH PARTICULAR REFERENCE TO THE CONDI- TIONS OBTAINING IN PERNICIOUS ANEMIA

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Man, as is well known, is one of the few species whose blood cells are permeable to glucose. The permeability rate of human blood cells for glucose has been the subject of many investigations attempting to define the significant factors of this phenomenon. Ege (1) was the first to investigate the importance of the concentration of the glucose. With a low glucose concentration there rapidly occurred a balance between the glucose in the extracellular fluid and the watery phase of the blood cells; with a higher degree of concentration, the blood cells gradually became swollen, with a much slower inward diffusion rate (a contributing factor here, however, was a probable simultaneous outward flow of potassium from the blood cells).

These conditions were more thoroughly examined by Bjerring (2) who likewise found the rate of diffusion slower when the concentration of glucose was raised. He noted further that the diffusion rate for glucose gradually diminished to a degree greater than might be expected from Fick's law<sup>1</sup> as more glucose entered the blood cells. There is much to suggest that it is a question of a tightening of the pores of the blood cell membranes in the presence of glucose molecules, as a similar lowering of the diffusion rate has been observed when collodium membranes are employed.

Bjerring showed too that the inward flow of the glucose could be brought to a complete standstill by the addition of cyanide of mercury to the sus-

<sup>1</sup> Fick's law accounts for the rate of diffusion through membranes by the formula:

$$k \cdot t = \log \frac{C_v}{C_v - C_i}$$

when  $k$  is the constant,  $t$  the time,  $C_v$  the concentration of the investigated substance in the outer fluid, and  $C_i$  the concentration of the substance in the watery phase inside the cell membrane.

pension fluid. This same worker also investigated the importance of the temperature. He found a  $Q_{10}$ <sup>2</sup> value of about 2, working with temperatures between 20° and 30° C., a reading corresponding to that found by Ørskov (3) for glycerin and thio-urea.

Investigations relating to the individual variations in the permeability of the blood cells from different persons are few in number. Schiødt (4), who has devised a formula for the estimation of the permeability constant by hematocrit estimation, found a uniform constant in relation to the permeability to  $\text{NH}_4\text{Br}$  in a series of normal men. He noted that the time interval during which the blood had stood prior to the performance of the test was a matter of importance, for example, older blood showed a lower permeability rate.

Schönheyder (5), using Schiødt's equation and the hematocrit method, tested the permeability constant of the blood cells for malonamid in 50 healthy and sick persons, and found a like constant in all the cases. The patients examined included various types of anemia, one case of pernicious anemia and one of hemolytic jaundice. No mention is made of the importance of the time interval during which the blood had stood before the performance of the tests. The experiments conducted by Jacobs (7) and by Höber and Ørskov (8), concerning the lapse of time before hemolysis took place in a suspension of human blood in solutions of different anelectrolytes were few in number. Furthermore, hemolytic tests are more uncertain than the determination of the permeability by the alterations in cell volume, so that their experiments perhaps bear no very great weight upon the subject in question.

Thus the literature to date affords no sure evi-

<sup>2</sup>  $Q_{10}$  stands for the increase in diffusion rate which is brought about by a rise in temperature of 10° C.

dence of a variation in the permeability of the blood cells from different persons.

Ørskov's experiments (6), however, establish the fact that there are considerable differences in the permeability to glycerin exhibited by the blood cells of different rabbits, while the permeability to thio-urea is more or less constant. It therefore seemed possible that the conditions relating to ammonium bromide and malonamid in the case of human beings might resemble those for thio-urea in rabbits, and that one might succeed in finding substances exhibiting individual variations in permeability rate towards the blood cells of different persons, similar to that shown for glycerin in the case of rabbits' blood cells.

Tentative investigations revealed a variation from person to person in the diffusion rate for glucose through their respective blood cell membranes. The technique followed, of which an account has been given in a previous communication (3), was the same as that employed in corresponding experiments with rabbits' blood. Concerning the principle of the method, the technique, and the determination of the permeability constant, reference may be made to the publication in question. Here we shall only mention certain details.

The blood to be used is taken from a vein in the arm, defibrinated in a small closed test tube and filtered. The test is first made after a lapse of 4 hours or more, 1 cc. of blood being carefully suspended in 100 cc. of a 0.88 per cent NaCl and 0.15 per cent NaHCO<sub>3</sub> solution, which is in equilibrium with oxygen that contains 4.7 per cent CO<sub>2</sub> and has therefore a pH value of 7.3.

The container, in which the permeability rate is estimated, is large enough to hold 30 cc. of the suspension. The glucose solution employed is 2 molar (it contains also 0.9 per cent NaCl), 1 cc. being taken for mixing with the blood cell suspension, so that this latter eventually contains 1.16 per cent glucose. For the purpose of gauging the permeability curve, adjustment curves are charted showing the effect of adding 1 cc. of 3.97 per cent saline solution to fresh samples of the blood cell suspension, whereby the osmotic pressure is raised half as much as with the addition of the glucose solution. In this manner the time required for the concentration of the glucose in the watery phase of the blood cells to reach half of

that found in the extracellular fluid is determined.

In the following pages the permeability rate is expressed as the minute constant ( $M-C$ ),<sup>3</sup> which is calculated according to Fick's law. A number of tests were carried out for the purpose of defining which factors are those influencing the permeability.

As already stated, Ege's and Bjerring's experiments showed that the concentration of the glucose plays a part in determining the rate of permeation. This was also the case in the experiments described below: the  $M-C$  became less with a higher glucose concentration.

	Glucose concentration in blood cell suspension per cent	Minute constant
Blood from normal person....	0.58 1.16	0.57 0.24
Blood from normal person....	0.58 1.16	0.78 0.33
Blood from patient with pernicious anemia.....	0.58 1.16	1.61 0.81

Thus it is seen that the permeability is raised 2.5 times, when the glucose concentration is halved.

In addition, the  $Q_{10}$  of the blood from 3 persons, 2 of whom were suffering from pernicious anemia and one was normal, was determined. The variations in the temperature were produced by passing water at different temperatures through the spiral glass in the container holding the blood cell suspension.

	Temperature range °C.	$Q_{10}$
Blood from normal person.....	12 to 22	5.0
Blood from patient with pernicious anemia.....	11 to 21	5.5
Blood from patient with pernicious anemia.....	11 to 21	6.25

These experiments gave, therefore, a resulting  $Q_{10}$  varying from 5.0 to 6.25, i.e., values which are considerably higher than those obtained by Bjerring. To ascertain if the reason for this divergence might lie in the utilization of different suspension fluids, a further series of tests was

<sup>3</sup> The constant calculated from Fick's formula is called the minute constant ( $M-C$ ), when the time unit is one minute. A high  $M-C$  reading corresponds with a rapid diffusion, a low  $M-C$  reading with a slow diffusion.

carried out with Christensen-Warburg's fluid,<sup>4</sup> and the same  $Q_{10}$  obtained as in the aforementioned experiments. Bjerring used higher degrees of glucose concentration (2 to 4 per cent) than those employed in the above experiments, and it is possible that this factor may account for the considerable differences in the resulting readings.

The experiments recorded in this paper were performed at a temperature of about 20°, and the constants obtained have been corrected to 20°, being calculated for a  $Q_{10}$  of 5.5, except in those instances in which the  $Q_{10}$  is directly estimated. The interval of time before the blood is used for the test undoubtedly plays a part in relation to the minute-constant; glucose diffuses through the blood cell membranes more readily when the blood employed is fresh than when it has stood for some hours. In the present series of experiments, the blood had stood for 4 hours or longer prior to its being used. After such an interval there occurs only very slight further alteration in the permeability judging from the few control tests performed. In this connection, however, there is a source of error, inasmuch as a control test was not performed in every case to determine how much the permeability had changed since the withdrawal of the sample or how much it might have changed if the blood had been allowed to stand for a still longer period.

The individual variations determined in the permeability to glucose, which were moreover much less than those shown in the case of glycerin and the blood from different rabbits, would have been more reliable if it had been possible in some way to stabilize the permeability of the blood cells immediately after the withdrawal of the blood. Preliminary tests, performed with this aim, did not, however, lead to any satisfactory result. Until further evidence is forthcoming, therefore, the best method of procedure without any doubt is to allow the blood to stand for so long a time that its permeability becomes more or less constant.

The permeability of the blood cells to glucose was investigated in 12 normal persons (Table I). The minute-constant here is seen to have varied within quite narrow limits, viz., 0.20 to 0.32, giv-

TABLE I  
*Normal persons*

Case number	Sex	Age	Glucose M-C
		<i>years</i>	
3	M	20	0.30
11	M	29	0.26
12	M	35	0.32
27	M	20	0.20
30	M	69	0.20
38	F	12	0.24
63	M	8	0.32
132	M	34	0.31
133	M	21	0.24
134	M	23	0.29
135	M	22	0.29
136	M	21	0.27
Total 12		Average	0.27
		Maximum	0.32
		Minimum	0.20

ing an average of 0.27. In 2 normal persons the permeability was tested at intervals of some days (Table II) and showed only small divergences

TABLE II  
*Normal persons on different days*

Case number	Date	Glucose M-C
12	December 13	0.33
	December 17	0.30
	April 4	0.33
	Average	0.32
132	January 10	0.35
	March 4	0.30
	March 7	0.28
	March 9	0.28
	March 10	0.31
	March 11	0.32
	March 16	0.30
	Average	0.31

from the average in these same, namely, a maximal variation of plus 0.04 and minus 0.03, i.e., roughly 13 per cent.

The alteration in the permeability of rabbits' blood cells to glycerin after venesection, shown by Ørskov (6), prompted the experiments recorded in Table III in relation to the permeability of the blood cells to glucose in the case of human beings in the regenerative stage after gastro-intestinal hemorrhage. In this series, the same average M-C was found as in normal persons, viz., 0.27. The range of variations on the other hand is seen to have been larger, namely, a maximal plus 0.12 and minus 0.12. The variations do not appear to

<sup>4</sup>Christensen-Warburg's fluid contains 11.5 grams  $\text{Na}_2(\text{COO})_2$ , 0.315 gram  $\text{KH}_2\text{PO}_4$ , and 1.365 grams  $\text{Na}_2\text{HPO}_4$  per liter.



TABLE III  
Posthemorrhagic anemia

Case number	Diagnosis	Hemoglobin	Red blood cells	Color index	M-C
		per cent	million		
1	Ulcer of duodenum; hematemesis; leukopenia	41	2.1	1.0	0.39
4	Duodenitis; hematemesis	61	3.3	1.0	0.26
5	Hematemesis	95	4.75	1.0	0.24
21	Ulcer of duodenum; melena	48	2.5	1.0	0.20
26	Cirrhosis of liver; hematemesis	37	2.7	0.7	0.23
29	Ulcer of duodenum; hematemesis	59	2.7	1.1	0.24
37	Hematemesis	41	2.1	1.0	0.15
59	Ulc. ventriculi	34	1.9	0.9	0.35
70	Hemorrhoid tumor; melena	41	3.5	0.6	0.35
Total			Average		0.27
9			Maximum		0.30
			Minimum		0.15

stand in any dependent relationship to the degree of anemia.

Thus a minute-constant of 0.40 or over was not found in association with posthemorrhagic anemia. This figure too is much above that of the readings obtained from normal persons. A minute-constant above 0.40 was, however, regularly noted in cases of untreated pernicious anemia, such as will be described in greater detail in the subsequent pages. It was a matter of interest therefore, to ascertain if similar conditions in relation to the permeability are present in other conditions. The provisional observations from such an investigation are recorded in Table IV, from which it is seen that in the large majority of the patients examined the *M-C* was below 0.40 and in most instances within the range of the readings obtained from normal persons. (A point of special interest was the finding of normal values in 2 cases of leukemia, Cases 42 and 43.)

An increased permeability to glucose, i.e., *M-C* over 0.40 was found in 2 patients. The one case, Number 28, was that of an *osteitis deformans* (particularly of the pelvic bones) complicated by a mild degree of anemia with hemoglobin 89 per cent, red blood cells 4 million. Here the *M-C* persisted practically unaltered, while the anemia was cured with iron (hemoglobin 99 per cent, red blood cells 5.07 million). The other case, Number 50, concerned a young man with diabetes in whom the control of the blood sugar presented certain difficulties. In this patient it was not possible to define the presence of any other abnormality, particularly no anemia. However, in two other cases of diabetes, Numbers 21 and 35, we obtained normal readings of the *M-C* for glucose.

The maximal variation between two tests from the same individual performed at different times (Table II) amounted to 25 per cent of the lowest values read. Among the 12 normal persons (Table I) the greatest variation was one of 60 per cent of the lowest reading, and among the cases of posthemorrhagic anemia (Table III) one of 160 per cent. If the patients with miscellaneous diseases be considered, apart from the two cases mentioned above in which the *M-C* was above 0.40, the maximal difference in this group was one of 124 per cent. The wide divergences seen in Tables III and IV certainly cannot be explained as technical errors, but must be interpreted either as individual variations or as alterations of permeability occasioned by disease. With regard to the smaller variations to be noted in Table I the question as to whether we have to take individual differences into account must for the present remain undecided.

In contrast with the conditions obtaining in simple posthemorrhagic anemia we found, as stated, a strikingly increased permeability of the red cells from untreated cases of pernicious anemia. From Table V it is evident that in all 4 cases the *M-C* was increased, ranging from 0.42 to 1.14 and with an average of 0.82, while the patients with posthemorrhagic anemia, as well as the normal persons, presented a *M-C* averaging 0.27 with a

TABLE IV  
Miscellaneous

Case number	Diagnosis	Glucose M-C
7	Hypertonia; meningeal hemorrhage	0.19
17	Hepatitis acuta	0.28
18	Hyperthyroidism	0.33
21	Diabetes mellitus	0.20
28	Osteitis deformans	0.42
31	Pneumonia	0.21
32	Undulant fever	0.20
33	Chorea	0.22
34	Arthritis; anemia	0.18
35	Diabetes mellitus	0.26
36	Gastritis	0.30
42	Acute myeloid leukemia; anemia	0.23
43	Acute myeloid leukemia; anemia	0.30
48	Hypothyroidism; anemia; epilepsy	0.26
50a	Diabetes mellitus prior to insulin	0.51
50b	Diabetes mellitus after insulin, on same day	0.51
50c	Diabetes mellitus after insulin, 9 days later	0.44
51	Cirrhosis of liver; anemia	0.21
60	Sequelae to thrombopenia	0.38
62	Chronic nephritis; puerperium; anemia	0.32
65	Morbus cordis; hypertonia; anemia	0.19
97	Acute hepatitis	0.17

maximal reading of 0.39. The rise in the permeability is seen to stand in no simple relationship to the degree of the pernicious anemia as expressed by the hemoglobin percentage, red cell count and color index. Thus the highest *M-C* reading was 1.14 and the lowest 0.42, with about the same degree of anemia in each case, viz., respective hemoglobin percentages of 37 and 35 and red cell count of 1.45 and 1.33 million.

TABLE V  
*Pernicious anemia untreated*

Case number	Hemo- globin	Red blood cells	Color index	Glucose <i>M-C</i>
	<i>per cent</i>	<i>million</i>		
2	72	2.65	1.35	0.92
15	37	1.45	1.28	1.14
88	23	0.74	1.55	0.81
100	35	1.33	1.32	0.42
Total 4			Average Maximum Minimum	0.82 1.14 0.42

In the other 6 cases of pernicious anemia<sup>5</sup> the permeability tests were performed after the commencement of specific treatment (Table VI).

TABLE VI  
*Pernicious anemia during treatment*

Case number	Hemo- globin	Red blood cells	Color index	Glucose <i>M-C</i>
	<i>per cent</i>	<i>million</i>		
8	76	3.1	1.23	0.81
9	82	4.0	1.02	0.21
23	70	2.9	1.21	0.46
44	81			0.20
46	81	4.1	0.99	0.48
49	46	1.5	1.53	0.72
Total 6			Average Maximum Minimum	0.48 0.81 0.20

Two of these gave the normal readings (*M-C* 0.20 and 0.21) and in the remaining 4 cases an increased reading (*M-C* 0.46 to 0.81). In these 6 patients, just as in the untreated cases, no definite parallelism was found between the degree of anemia and the value of the permeability read.

<sup>5</sup> We offer our thanks to Dr. K. H. Krabbe, Physician-in-charge of the Department for Nervous Diseases, Kommunehospital, for the opportunity to examine Cases 44, 46 and 49.

The observation that two of these patients exhibited normal permeability suggests that the specific treatment was responsible for the effect. More detailed information on this point is furnished by the accompanying curves which show the course of the anemia and of the permeability during treatment.

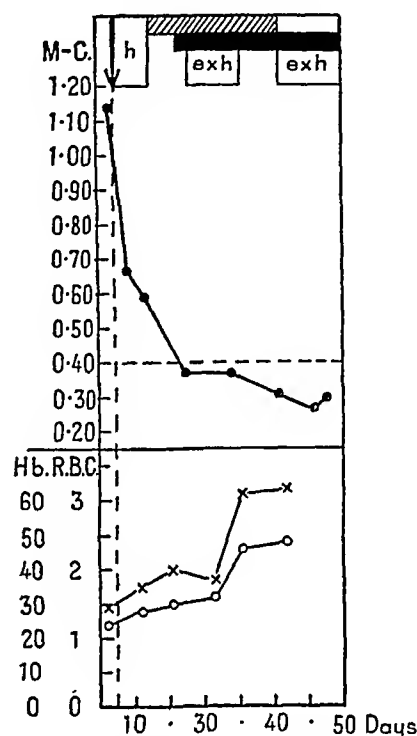


FIG. 1. CASE 15 (TABLE V). NOT PREVIOUSLY TREATED.

*Treatment.* Hepsol 4 cc. daily intramuscularly (h); tonicum "DAK" 10 cc. t.d. (hatched); exhepa 1 dose t.d. (exh.), reduced iron 0.5 gram t.d. (solid black).

The upper curve in each Figure indicates the *M-C* drawn ●—●, the lower curve the hemoglobin percentage shown by ×—×, the red-cell count in millions ○—○, and the reticulocyte percentage ●····●; the commencement of the specific treatment is indicated by an arrow. The abscissae show the number of days after admission to hospital.

Characterizing these curves is the pronounced decrease in the permeability readings which assumed entirely normal values during the treatment in every case.

This decrease, however, is lacking in Figure 5. This patient had been undergoing treatment over a long period prior to admission. In this instance the permeability rate was normal from the beginning of the examination and continued so.

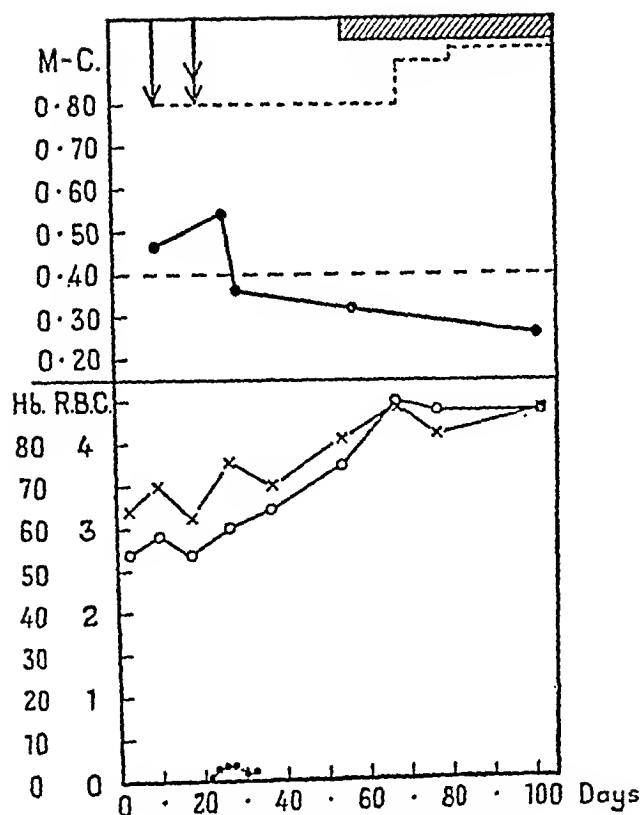


FIG. 2. CASE 23 (TABLE VI).

Until just before admission to hospital the patient had been receiving liver by mouth and by injections.

*Treatment.* Hepsol 4 cc. daily (from single to double arrow); campolon 2 cc. daily, later every second and third day (from double arrow); pylorin 10 grams t.d. (hatched).

Further, it is to be noted that the permeability returned to normal in all the cases where there was still a definite and in most instances a considerable degree of anemia present. In these preliminary experiments it has not been possible to carry out observations over any length of time during the untreated stage of the disease. The fall in the *M-C* value before treatment, seen in Figures 6 and 7, is so small that it is not necessarily a genuine one. The possibility, however, cannot be excluded that rest in bed and the hospital diet among other factors may have had some action upon the permeability. In Figure 6 the permeability curve shows a gradual regular fall from values bordering on the normal to completely normal readings. Figure 7 on the other hand shows a sharp acceleration in the fall of the permeability immediately after the beginning of treatment. Since a similar fall occurs in Figures 1 and 3, it may be maintained with some conviction that the fall was dependent upon the specific treatment.

In Figure 7, there occurs a depression upon the permeability curve corresponding to the rise in the number of reticulocytes. It is possible that the reticulocytes, amounting in this instance to 49 per cent of the circulating blood cells, are cells with a particularly low permeability to glucose. No weight, however, can be placed upon this observation unless it be possible to reproduce it.

Tests were conducted in relation to the permeability rate for glycerin, thio-urea and malonamid in fewer instances than the estimations of the permeability rate for glucose. In spite of the small amount of material investigated, these tests furnished very interesting observations, so that we shall therefore give a short account of the result obtained.

With regard to *glycerin* it was noted that the permeability rate was lower in pernicious anemia than in normal persons and that the administration of liver did not produce any alteration in the rate (one case only examined, Case 15, Figure 1, in which, however, there was observed a striking effect upon the permeability rate for glucose). A

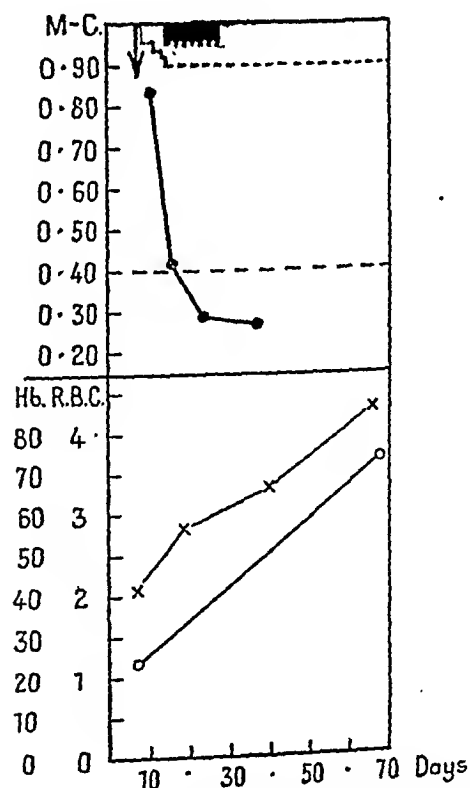


FIG. 3. CASE 49 (TABLE VI). NOT PREVIOUSLY TREATED.

*Treatment.* Pylorin 10 grams once daily (from arrow); hepsol 4 cc. increasing to 10 cc. daily (stippled); betaxin 2 cc. on alternate days (solid black).

certain degree of conformity was shown in a series of cases between the permeability rates for thio-urea and for glucose. Concerning thio-urea there were noted quite large individual variations which in their broad features resembled those ob-

exhibited a normal permeability rate at the end of 20 days' treatment.

In our series of cases of pernicious anemia, the diagnosis rested upon the general examination and the blood picture, supplemented by repeated test meals, as well as by the reaction to specific treatment (in Case 8, Figure 4, and Case 9, Figure 5, this reaction was confirmed by the response shown during previous admissions to hospital). Marked paresthesia was exhibited by Cases 8, 9, 23, 88 and 100, and characteristic myelopathies were noted in Cases 23, 44 and 49. In Case 15, Figure 1, x-ray examination revealed changes in the stomach which might be interpreted as a carcinoma of the pylorus. The pernicious anemia in this case must therefore be regarded as having been probably complicated—if not occasioned—by a carcinoma of the stomach. The patient has since died from cachexia with severe ascites (no autopsy).

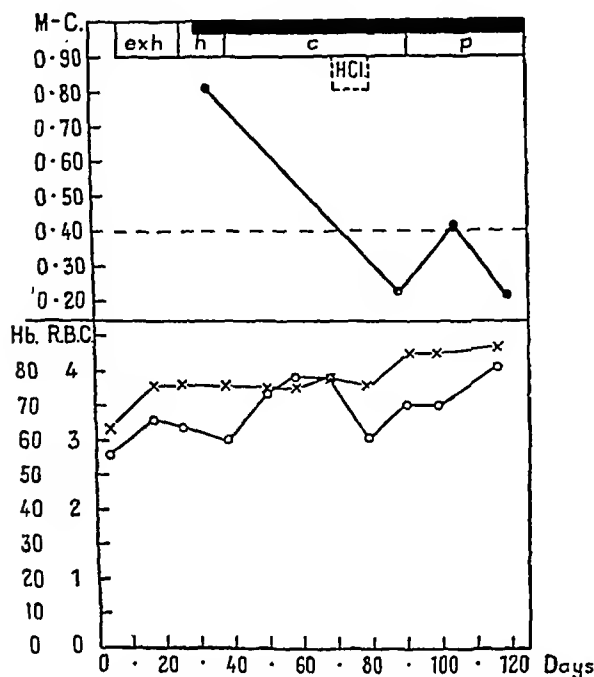


FIG. 4. CASE 8 (TABLE VI). TREATED WITH LIVER FOR ABOUT 12 MONTHS UP TO THE TIME OF ADMISSION.

*Treatment.* Exhepa 1 dose t.i.d. (exh.); hepsol 4 cc. daily (h); campolon 2 cc. daily (c); pylorin 10 grams b.i.d. (p); reduced iron 0.5 gram t.d. (solid black); hydrochloric acid 20 drops t.d. (HCl).

served for glucose. Two cases of pernicious anemia showed at first a permeability rate for thio-urea which was twice as high as the normal figure, a difference which disappeared during the course of treatment.

Concerning malonamid a series of patients exhibited a constant permeability rate showing a deviation of less than 10 per cent from the average reading. Two cases of pernicious anemia with an increased permeability to glucose were studied, and an increased permeability rate for malonamid was found. In the one patient (Case 23, Table VI, Figure 2) the increase was one of 15 per cent above the previously mentioned average value, and in the second case (Number 15, Table V, Figure 1) an increase of 42 per cent. This latter case

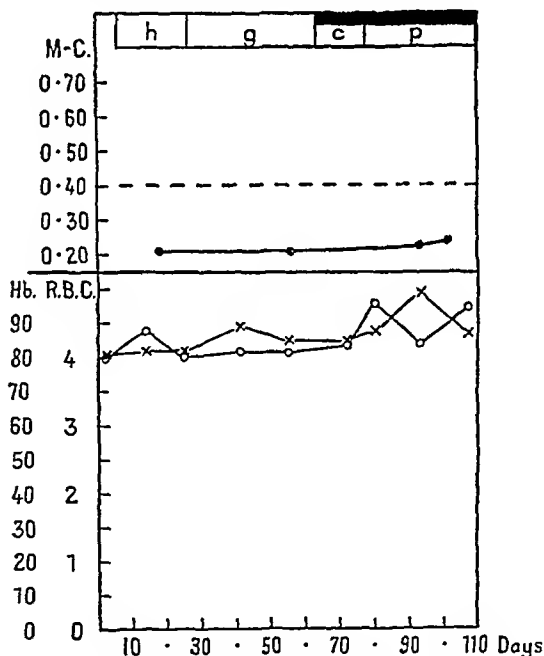


FIG. 5. CASE 9 (TABLE VI). TREATED WITH VENTRICULIN UNTIL ADMISSION.

*Treatment.* Hepsol (h); Ext. hepatis "GEA" (g); campolon (c); pylorin (p); iron (solid black).

In making any attempt to explain the increased permeability of the blood cells in pernicious anemia (to glucose, malonamid and thio-urea) we stand upon very uncertain ground. The size of the blood cells does not explain the increased

## SUMMARY

The extent and nature of the variations in the permeability of the red blood cells in different individuals has been studied with special reference to their permeability to glucose. Some of the factors exerting an influence upon the permeability are examined in detail.

1. By increasing the concentration of glucose in the suspension fluid from 0.58 per cent to 1.16 per cent the permeability is reduced 2.5 times.

2. In the performance of the experiments in question the  $Q_{10}$  was 5.0 to 6.25.

3. The variations in the normal permeability to glucose were found to exceed the limits of any technical error, being probably an expression of individual differences.

4. In posthemorrhagic anemias, comparatively large variations were noted without any relationship to the degree of the anemia, and no increased permeability was found.

5. Entirely similar variations were found in a series of miscellaneous diseases.

6. In one case of osteitis deformans and in one among three cases of diabetes a definitely increased permeability was noted.

7. Ten cases of pernicious anemia were examined. In 4 untreated cases, the permeability was markedly increased (to as much as 4 times the average normal reading), while the same conditions were noted in 4 out of 6 cases tested during the specific treatment.

8. In 7 cases of pernicious anemia the permeability was tested periodically during the course of treatment, during which it became normal in each case.

9. The effect of the specific treatment upon the permeability became demonstrable early and in 2 instances was noted to begin after 1 to 3 days' treatment. In all the cases, normal readings were obtained at a time when a high degree of anemia still persisted.

10. Individual examinations of the permeability to malonamid and to thio-urea yielded results similar to those observed in the case of glucose, while the permeability to glycerin was found to be normal in pernicious anemia, and to be unaffected by specific treatment.

11. The possible mechanism of the increased permeability in pernicious anemia and the effect of specific treatment are discussed.

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# THE INFLUENCE OF MUSCULAR EXERCISE ON BLOOD SUGAR CONCENTRATIONS

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The beneficial effects of exercise on certain types of diabetes mellitus have long been recognized. Occasionally a young and active diabetic, with a stable glycemia, controlled by diet and insulin, develops severe and sometimes prolonged insulin reactions following participation in strenuous sports; while other young and equally active diabetics, with an unstable glycemia, find participation in active sports a burden, accompanied by glycosuria and general malaise.

The effect of exercise on glycosuria and glycemia was determined in twelve cases of diabetes mellitus treated with insulin; in four of diabetes mellitus treated without insulin; in three of renal glycosuria; in two of hypotension; in one patient with glycosuria of pregnancy, another with obesity, a third with epilepsy, and in three normal individuals.

## REVIEW OF LITERATURE

The first direct observation on the changes in the blood sugar resulting from muscular exercise was that of Chauveau and Kaufmann quoted by Strandell (1), who demonstrated in 1886 that the blood sugar in the vein leading from the masseter muscle of a horse decreased when the horse chewed. Strandell in his historical review of the subject says that the first studies on man were made by Weiland in 1908, when he proved that the venous blood sugar concentration was considerably lower after exhausting exercise in Gärtner's ergostat than before the exercise. This was done by measuring the blood sugar concentration before the exercise was started and again after it was stopped.

Although Lichtwitz (9) in 1914 determined the effect of exercise on diabetic subjects, which will be discussed later, most of the work was done on normal subjects. Gordon, Kohn, Levine, Matton, Scriver and Whiting (2) report a study of the blood sugar concentrations in marathon runners during 1924 and 1925. They found that the runners in bad condition at the finish of the race invariably had low blood sugars, values which could be classed as hypoglycemic. The runners trained on high carbo-

hydrate diets prescribed by the authors and those runners who, while running, ate glucose in some form finished in better condition than those who did not; and their blood sugars were not low enough to be classified as hypoglycemic. Best and Partridge (3) also reported hypoglycemic blood sugar levels in three of ten marathon runners following exercise. Wollmer (4) noted a decrease in the blood sugar in normal and obese persons following muscular exertion. He describes, however, immediately after the start of the work, a short but sharp rise in the blood sugar level which is followed by a decline. This is noted particularly when the work is heavy—more than thirteen kilogrammeters per second—according to his experiments. He noted no difference in the blood sugar curves in normal and obese persons. Other workers have described a rise in the blood sugar level following work. Edwards, Richards and Dill (5) found on examination of a number of football players that hyperglycemia is uncommon in exercise unless it is accompanied by emotion, as on the football field. The blood sugar reaches a peak about one-half way through the game—one varsity player had a blood sugar of 244 mgm. per 100 cc. after thirty minutes of play. Schneider (6), in examining athletes at Innsbruck, described an increase in the blood sugar following a fifty meter dash as compared to that before the race, in all of the participants who were examined. This occurred though the pre-race values varied in the different contestants between 50 and 153 mgm. per 100 cc. These differences in the blood sugar concentrations by the various authors have been considered to be due to the differences in amount as well as the intensity of the exercise. Generally, the blood sugar is lowered in long exhaustive exercises, sometimes markedly so; while the opposite effect is often found in exercise of short duration (1).

The effect of training on the blood sugar levels during exercise has also been studied. Hofmann (7) demonstrated that during exercise the blood sugar remains at a more constant level in well trained subjects than in subjects not in training. Effects from emotion were not evident in his experiments as they were in those of Edwards and Dill previously mentioned.

As has been mentioned, Lichtwitz (9) determined the effect of muscular exercise on diabetic subjects. He found that the blood sugar usually decreased but may increase with exercise, such things as severity of the diabetes and training influencing the direction of the blood sugar change. He noted particularly that in severe diabetes mellitus the values were more frequently higher than they were lower after exercise than before exercise. In one case reported (Number 19) the resting

<sup>1</sup> Data taken from a dissertation submitted to the graduate faculty in candidacy for the degree of Master of Science, Department of Medicine, University of Chicago, 1936.

blood sugar value was 275 mgm. per 100 cc., and following exercise it was 377 mgm. per 100 cc. The lactic acid in this case had also risen markedly to a value of 112.5 mgm. per 100 cc. The possibility of lowering the blood sugar by exercise in diabetes mellitus has been known for some time. Joslin has pointed out that glycosuria in mild cases of diabetes mellitus may be controlled by regulating exercise taken after meals. Lawrence (10) reports lowering of the glucose concentration of the blood and the appearance of hypoglycemic reactions in two cases of diabetes mellitus following exercise. He also points out the supplementary effect of exercise in exaggerating the action of insulin. Gerl and Hofmann (11) found that the blood sugar decrease was too great to be explained by mere summation of the effect of insulin and exercise. Hamburger (12) in a report of the literature and in one case of his own showed how physical exercise (walking) permitted excellent control of the glycosuria in a child with diabetes mellitus.

Theories offered in explanation of the increase in the blood sugar following exercise are of interest here. Grott, Kowalski et al. (8) conclude that the elevation of the venous blood sugar after exercise depends upon the outflow of the sugar stored in the liver and muscles (which they call the central and peripheral reservoirs); and upon the rate of carbohydrate consumption by the tissues during work. Edwards, Richards and Dill (5) think the emotional factor is the chief cause of the blood sugar elevation following exercise. Bruusgaard (13) also mentions this as a possibility but points out that he has found hyperglycemia following exercise in which there has been no emotional excitement.

Sóskin et al. (14) concluded from their experiments that there was no evidence of significant improvement in the diabetic tolerance or a decrease in the insulin requirements as a result of physical training over an extended period.

Sometime after this work was started and while it was still in progress, Richardson (15) published a study of the effect of exercise on the blood sugar in sixty-one diabetics.

He was able to divide his series into two groups according to the level of the fasting blood sugar. In the first group, mild diabetics with fasting blood sugars below 175 mgm. per 100 cc., exercise produced a marked lowering of the blood sugar. The second group composed of severe diabetics with fasting blood sugars over 175 mgm. per 100 cc. reacted to the same amount of exercise by an elevation of the blood sugar above that of the fasting level. He also found that intravenous insulin when given before a period of rest in as small quantities as 0.1 unit was without marked effect on the blood sugar, but when given immediately before a period of exercise caused a recognizable drop in blood sugar. He also describes a more marked lowering of the blood sugar following exercise after food has been taken than from the same amount of exercise taken without food.

## EXPERIMENTAL PROCEDURE

The experimental procedure consisted of determining the sugar concentration of capillary blood at frequent intervals—usually every fifteen minutes—for a period of three to ten hours following the ingestion of a mixed meal or a dose of glucose while the subject was inactive; and again determining the glucose concentration of the capillary blood of the same patient at the same time intervals following an identical meal, while the subject performed a measured amount of work. Usually four or more experiments were made on each subject and each experiment was started in the morning at the subject's customary breakfast hour.

Determinations of blood sugar were made on capillary blood taken from a finger and estimated by the method of Folin and Svedberg (16). This method was modified by collecting the blood in one inch funnels on which the stems had been fused and removed. Three drops of 0.5 per cent potassium oxalate solution were air dried in the bottom of the funnels; this was sufficient to prevent clotting in as much as 0.3 cc. of blood without causing hemolysis in lesser amounts. This micro blood sugar method is accurate for concentrations ranging from 40 to 350 mgm. per 100 cc. Checks on venous blood were frequently made by macro determinations by the method of Shaffer and Hartmann (17).

The urine was analyzed for sugar qualitatively by Benedict's solution, and when necessary quantitatively by the Shaffer-Hartmann titration method, at intervals of thirty to sixty minutes throughout the experimental period. Subjects with glycosuria brought to the laboratory urine specimens collected during the preceding twenty-four hours. These were titrated for glucose which had previously been identified by fermentation tests. The urine was tested for acetone by the nitroprusside reaction.

Each case of diabetes mellitus and the case of epilepsy received a mixed meal identical with that given as part of their regular therapeutic regime in place of a glucose test meal. Those cases taking insulin received their usual dose, and care was taken to keep the time between the injection of insulin and the beginning of the meal constant. Non-diabetic subjects, with the exception of



Cases 21 and 24, and the diabetic case Number 13, received glucose test meals containing an equal amount of carbohydrate. The glucose was given diluted to four hundred cc. with water and flavored with lemon juice.

During the resting periods, except when blood was being taken, the subject had the freedom of the laboratory, but usually spent his time in sitting quietly reading or conversing with the workers.

All exercise was performed on a stationary bicycle, the amount of work being measured by an ergometer of the Sevringhaus type (18). The interval between the ingestion of food and the beginning of exercise was kept fairly uniform. Exercise was never begun after a meal of glucose until the blood sugar had begun to rise, to make more striking if possible, the ability of work to lower the concentration of blood sugar. It soon became evident that two subjects of normal weight for height, and of the same age, could not accomplish equal amounts of work with comfort. Such factors as previous training, musculature, and nervous reactions were observed carefully, and overambitious subjects were advised against exhaustion. No standard amount of exercise was imposed. Each subject exercised at the rate and over a period of time that was agreeable to him. After exercise, the subject usually rested in bed for fifteen to thirty minutes.

The question whether or not the blood sugars were taken while the work was in progress, or whether work was stopped even momentarily while the blood was drawn, may arise in the reader's mind. Work was stopped from two to four minutes on every subject at the time the blood was taken. The time between stopping work and drawing the blood was, however, less than one minute, the remainder of the time being spent by the subject in rest. Some justification for this method may be necessary in view of Christensen's report in 1931 (19) that the blood sugar changed rapidly back to the pre-work levels following a period of work; as do the pulse, respiratory rate, and even the basal metabolic rate. He said, therefore, that unless the blood was taken while work was still in progress the blood sugar values were not those of work but were "restitution values." Although this is literally

true, in these experiments the blood sugar values shown on the graphs as those during work have been taken some few seconds after work was stopped. This was done for three reasons. First, with the amount of work performed by the subjects rest was necessary at fifteen minute intervals. It was decided, therefore, to take the blood as soon as possible after the subject had stepped from the stationary bicycle and walked three paces to a chair. This method simplified the drawing of the blood. Second, it was found that the difference between blood sugar levels during work, and from one to three minutes after stopping work, was not greater than the experimental error of the method. Also, if the blood sugar was descending during work the level a few minutes after work was stopped—Christensen's "restitution" value—was lower than the preceding value obtained during work; but if the blood sugar was ascending during work the level obtained a few minutes after work was stopped was higher than that level found while work was in progress. Third, if greater differences occurred than were found, it would not change the results of these experiments, since the attempt was being made to show only the relative changes in the blood sugar levels produced by work, and not necessarily the absolute values.

#### EXPERIMENTAL RESULTS

The experimental material is divided into five groups to simplify discussion.

Group I. Diabetes mellitus treated with insulin	
A. Well controlled (stable glycemia) .....	3 cases
B. Difficultly controlled (unstable glycemia) .	4 cases
C. Uncontrolled (unstable glycemia) .....	5 cases
Group II. Diabetes mellitus treated without insulin .....	4 cases
Group III. Renal glycosuria .....	3 cases
Group IV. Miscellaneous .....	
A. Hypotension .....	2 cases
B. Glycosuria of pregnancy .....	1 case
C. Obesity .....	1 case
D. Epilepsy treated with a ketogenic diet ....	1 case
Group V. Normal subjects .....	3 cases
Total .....	27 cases

Table I gives a brief summary of the subjects' histories and the experimental procedures; more detailed information concerning diet, insulin, and total work performed will be found on the individual graphs.



TABLE I  
Summary

Case number	Sex	Age	Height	Weight	Physical development	Family metabolic history	Average work	Duration of work
		years	cm.	kgm.			kilo-gram-meters per second	minutes

GROUP I. DIABETES MELLITUS TREATED WITH INSULIN  
A. Well controlled (stable glycemia)

1	M	29	173.5	56.8	Fair	None known	2.20	75
2	F	40	164.0	60.9	Fair	None known	2.40	45
3	F	23	156.5	51.0	Fair	Mother had goiter	2.20	45

## B. Difficulty controlled (unstable glycemia)

4	M	19	175.0	62.3	Good	None known	3.70	105
5	M	24	179.0	63.0	Good	Mother had goiter	2.20	135
6	M	39	172.0	63.8	Good	Glycosuria in one nephew	1.60	135
7	M	24	166.0	49.9	Fair	Glycosuria in one cousin	3.00	60

## C. Uncontrolled (unstable glycemia)

8	F	30	160.0	47.0	Poor	None known	1.10	75
9	M	13	126.0	28.0	Fair	None known	2.25	38
10	F	17	163.0	51.0	Fair	None known	1.85	53
11	M	25	171.0	67.5	Fair	Father has glycosuria	1.37	53
12	F	34	169.0	61.3	Good	Unknown	1.53	25

## GROUP II. DIABETES MELLITUS TREATED WITHOUT INSULIN

13	M	29	178.0	69.4	Good	None known	5.00	60
14	M	45	180.0	72.4	Good	None known	3.34	45
15	M	47	163.0	101.0	Fair	None known	3.65	45
16	M	30	167.5	64.6	Fair	Mother and two siblings have glycosuria	2.14	60

## GROUP III. RENAL GLYCOSURIA

17	M	47	168.0	77.4	Fair	Paternal grand-mother had glycosuria	3.02	60
18	M	34	166.5	63.1	Fair	Unknown	3.00	65
19	F	34	165.0	56.1	Fair	Glycosuria in mother and two siblings	0.80	60

## GROUP IV. MISCELLANEOUS

## A. Hypotension

20	F	22	157.5	47.2	Poor	None known	2.90	50
21	F	47	170.5	75.1	Poor	None known	0.0	

## B. Glycosuria of pregnancy

22	F	38	165.0	75.1	Fair	Glycosuria in mother	2.38	30
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## C. Obesity

23	F	27	156.0	89.7	Fair	One sibling had goiter	1.58	45
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TABLE I—Continued

Case number	Sex	Age	Height	Weight	Physical development	Family metabolic history	Average work	Duration of work
		years	cm.	kgm.			kilo-gram-meters per second	minutes

## D. Epilepsy treated with a ketogenic diet

24	M	19	162.0	46.3	Good	Paternal grand-mother had epilepsy	3.90	90
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## GROUP V. NORMAL SUBJECTS

25	M	26	174.0	79.0	Good	None known	2.87	75
26	M	24	183.0	79.6	Good	None known	3.84	45
27	M	29	176.0	76.3	Good	None known	4.30	60

One illustrating figure will be used in each group to demonstrate the changes in the blood sugar level produced by exercise.

## Group I. Diabetes mellitus treated with insulin

## A. Well controlled cases (stable glycemia).

The characteristics of this group are well shown by Case 1, Figure 1. They are for this group;

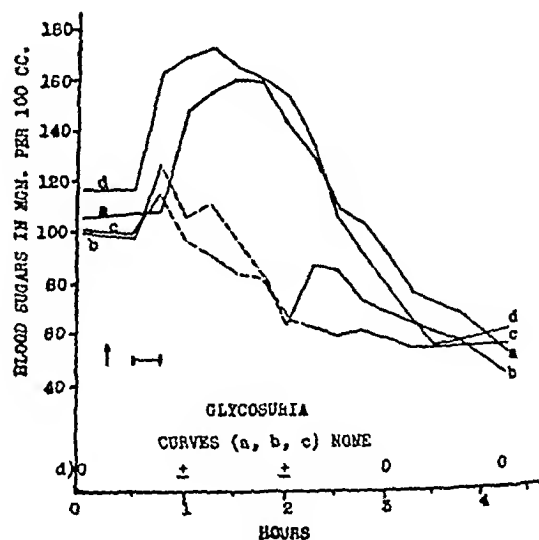


FIG. 1. CASE 1.

— Rest. --- Period of work. Work in kilo-gram-meters. Curve (b) 8,516. Curve (c) 10,954. |—| Mixed meal. Carbohydrate 48 grams, protein 20 grams, fat 64 grams, calories 848, glucose equivalent 66 grams. ↑ Insulin 15 units.

a rather low fasting blood sugar level which varies little from day to day, 86 to 150 mgm. per 100 cc.; a maximum postprandial blood sugar which is not a great deal higher than that of the

group of normal or non-diabetic individuals studied, 156 to 197 mgm. per 100 cc.; and the comparative ease with which exercise lowers the postprandial blood sugar. This reduction ranged from 12 to 90 mgm. per 100 cc. Glycosuria is not found in this group.

*B. Difficultly controlled cases (unstable glycemia).* These cases differ from those just described in the following respects; the greater daily variation of the fasting blood sugar level which varies from 96 to 232 mgm. per 100 cc.; the higher postprandial blood sugar levels of from 158 to 256 mgm. per 100 cc.; and usually the appearance of glycosuria sometime during the resting periods. Case 4, Figure 2, is a good example of this group.

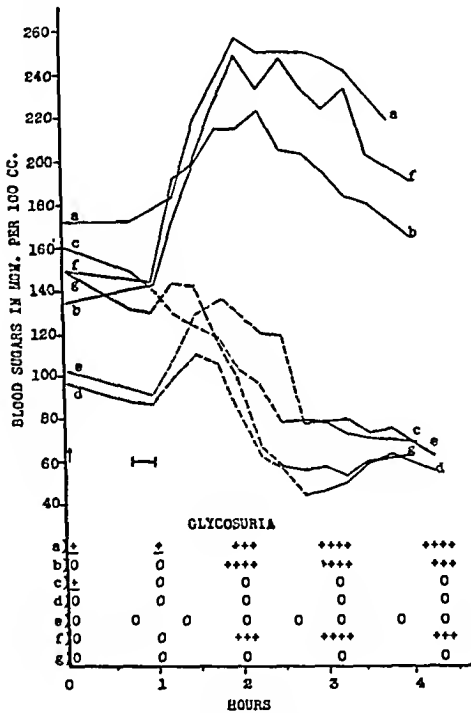


FIG. 2. CASE 4.

— Rest. --- Period of work. Work in kilogrammeters. Curve (c) 21,795. Curve (d) 26,986. Curve (e) 24,676. Curve (g) 19,034. |—| Mixed meal. Carbohydrate 61 grams, protein 25 grams, fat 50 grams, calories 794, glucose equivalent 82 grams. † Insulin 28 units.

It is noted in this group, comprised of Cases 4, 5, 6, and 7, that exercise is very effective in lowering the postprandial blood sugar, and from

the illustrative figure and the effects upon the individual subjects, such as the hypoglycemic symptoms experienced by all of the subjects in this group, the effects of exercise are more marked than in the group of well controlled subjects. The most striking difference between this and the preceding group of cases is the difference between the levels of the blood glucose at the end of the fourth hour during rest and work respectively. This difference varied from 56 to 170 mgm. per 100 cc., while in the preceding group the blood sugar levels at the end of four hours were all within 20 mgm. per 100 cc., regardless of whether the subject had exercised or rested. Although there was this marked difference between the final blood sugar values after rest and exercise, the final values of all the studies following rest and those following exercise varied as a rule less than 30 mgm. per 100 cc.

The work in this group of cases was not excessive when compared by the subjects to the swimming, tennis, golf, or camp life in which they indulge.

*C. Uncontrolled cases (unstable glycemia).* Four of these cases belong to a group which might be called the acute progressive juvenile type of diabetes mellitus. Though usually found in the younger age groups this type is not necessarily limited to it. The added factor of inability to follow dietary and insulin therapy prescribed, because of financial reasons or lack of will, complicates management in at least two of these cases—Cases 8 and 10. Adequate control in the remaining two cases, Numbers 9 and 11, was not satisfactorily achieved during this period of study. Case 12 is not offered as an example of the progressive type of juvenile diabetes. The onset of the disease was acute, but not progressive. Emotional factors complicate management to the extent of making the case uncontrolled.

Case 8, Figure 3, is taken as a typical example of the response to exercise by members of this group. The characteristics of the group, as shown by the blood sugar curves, are a high fasting level of 182 to 376 mgm. per 100 cc., with an abrupt fall in the blood sugar to 56 to 238 mgm. per 100 cc. in the fourth hour; a drop which, in four of the five cases, meant a change of blood sugar level from 200 to 260 mgm. per 100 cc. in

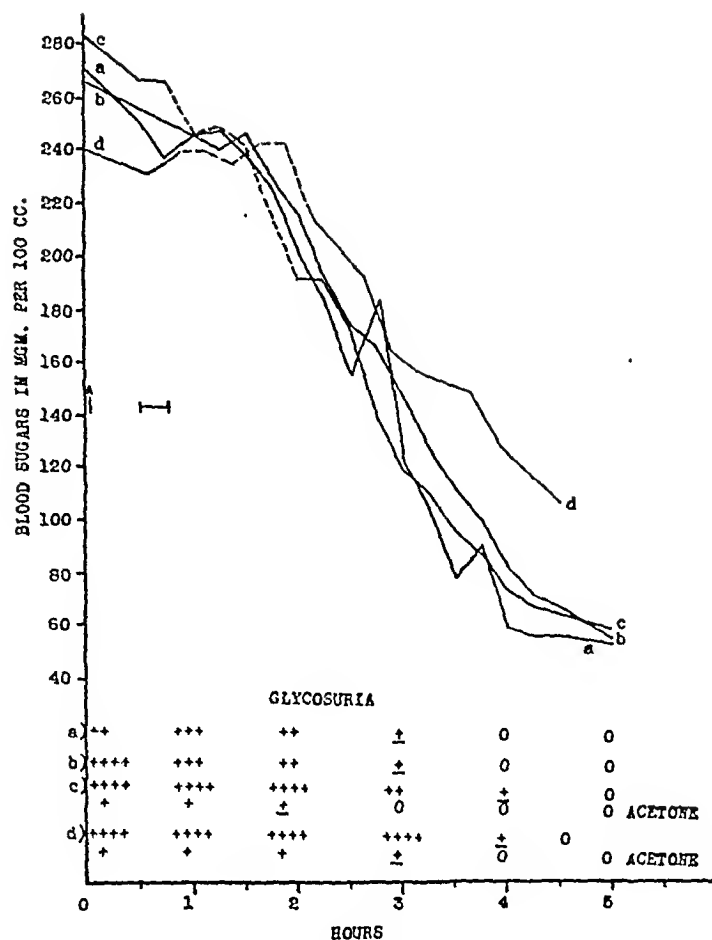


FIG. 3. CASE 8.

— Rest. --- Period of work. Work in kilogrammeters. Curve (c) 3,937. Curve (d) 6,688. |—| Mixed meal. Carbohydrate 28 grams, protein 14 grams, fat 50 grams, calories 618, glucose equivalent 41 grams. ↑ Insulin 25 units.

four to five hours every morning. This rapid fall in blood sugar occurred whether or not exercise was taken. The effectiveness of the insulin is demonstrated by the character of the curve. The effect of muscular exercise is difficult to determine as will be discussed later.

#### Group II. Diabetes mellitus treated without insulin

Four cases are included in this group; Cases 13, 14, 15, and 16; of which Case 13, Figure 4, will be used as the illustration. These cases present a number of interesting points. First there is a small fluctuation in the daily fasting blood sugar level, which, with the exception of one case, ranged between 91 and 122 mgm. per 100 cc. Second, there is a rather close similarity of comparable curves at any one time throughout the experimental period. Third, the effectiveness of

the muscular exercise on lowering the postprandial blood sugar is very similar to that found in the normal subjects. The above three characteristics are all much like those found in normal subjects under similar conditions. A fourth characteristic, demonstrated in this study only by Case 13, is the higher postprandial rise following the second meal of the day; a finding noted in diabetes mellitus and frequently utilized to diagnose mild cases which give a fairly normal glucose tolerance test.

The mixed meals fed these subjects, with the exception of Case 13, contained one gram of carbohydrate per kilogram of standard body weight. This represents approximately double the usual intake for each subject at the breakfast meal. The plan was adapted to deliberately provoke a hyperglycemia in the resting blood sugar curves as a control for the demonstration.

#### Group III. Renal glycosuria

Three complicated cases of renal glycosuria are included in this group. The complications are, hypertension, peptic ulcers, and pregnancy, for Cases 17, 18, and 19, respectively. Case 17, Figure 5, is used as the illustrative case. The only effect of the hypertension on this study was to limit to some degree the amount and speed of exercise that it seemed advisable to permit. These cases react to exercise for the most part as do normal individuals. The fasting blood sugar level is within the normal limits and fluctuates only little, apparently even less than the daily fluctuation found in the normal subjects by Holt and Greisheimer (20). Also the blood sugar curve is back at the fasting level in less than two hours, it goes through a hypoglycemic phase, and, in Cases 17 and 18, glycosuria appears only in the postprandial periods at levels of 140 to 160 mgm. per 100 cc., which is below the normal renal threshold. This type of response permits a diagnosis of a cyclic renal glycosuria according to the classification of Holst (21). Case 19 demonstrated the effect of insulin on renal glycosuria of a constant nature. The effect of exercise on this case is also very similar to that of the normal individual. The blood sugar was lowered very little since only a small amount of work was done.

These three cases are of particular interest

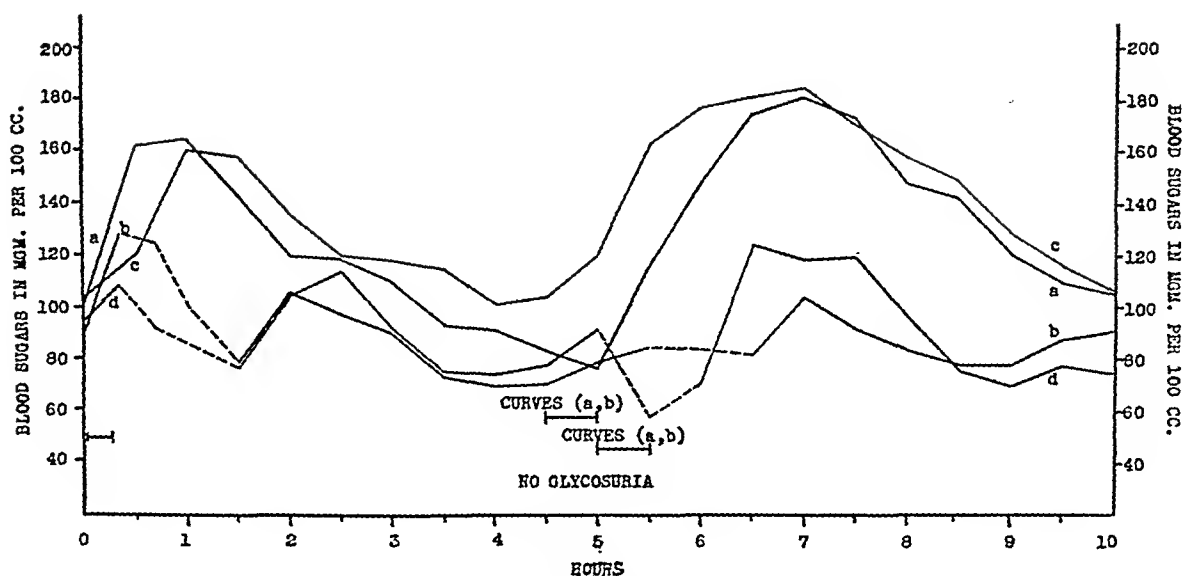


FIG. 4. CASE 13.

— Rest. --- Period of work. Work in kilogrammeters.

	First period	Second period
Curve (b) .....	17,599	18,530
Curve (d) .....	23,078	18,777

|—| Mixed meal. Carbohydrate 50 grams, protein 17 grams, fat 39 grams, calories 619, glucose equivalent 64 grams.

from the standpoint of a differential diagnosis between renal glycosuria and diabetes mellitus and are published elsewhere in more detail (24).

#### Group IV. Miscellaneous cases

*A. Hypotension.* The two cases in this group, Cases 20 and 21, were referred to the Nutrition Laboratory for blood sugar analysis because of symptoms suggestive of hypoglycemia; that is, weakness, inability to work, dizziness, and fainting, all relieved by the ingestion of food.

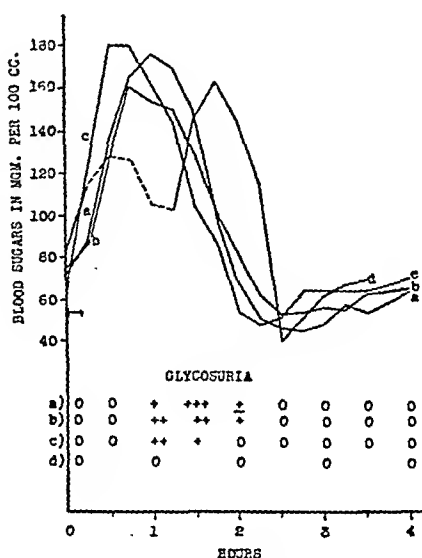


FIG. 5. CASE 17.

— Rest. --- Period of work. Work in kilogrammeters. Curve (d) 10,850. |—| Glucose test meal 70 grams.

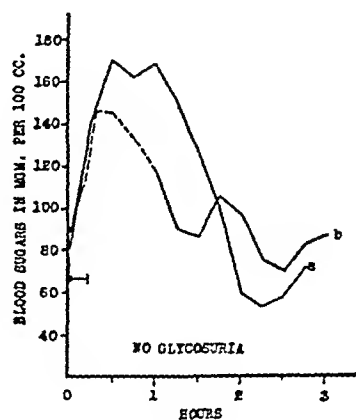


FIG. 6. CASE 20.

— Rest. --- Period of work. Work in kilogrammeters. Curve (b) 8,683. |—| Glucose test meal 50 grams.

With the subject (Case 20) in a resting state following the ingestion of a glucose test meal the blood sugar course followed a normal curve for one hour, Figure 6. At this point an abrupt drop occurred at a rate and to a level which frequently causes diabetic subjects to complain of insulin reactions. She complained of her usual symptoms of weakness, dizziness, and faintness with hunger at the time the blood sugar level was within the hypoglycemic range, 50 to 70 mgm. per 100 cc. When the glucose test meal was followed by exercise (Curve *b*) the absorption of the glucose appeared to be delayed as demonstrated by the second peak of the curve. During this experiment, the glucose content of the blood did not reach a hypoglycemic level, and there was no complaint of the symptoms of weakness, dizziness, etc. Insufficient exercise to be measured either by the ergometer or fall in the blood sugar level was done in Case 21 for purposes of comparison.

Simultaneous occurrence of hypoglycemic blood sugar levels and the complaints of weakness, dizziness and faintness relieved by food, suggest a diagnosis of hypoglycemia of unknown etiology in these two cases.

*B. Glycosuria of pregnancy.* This case, Number 22, was seen in the seventh month of her seventh pregnancy at which time a glucose tolerance test by macro blood sugar methods showed a typically diabetic type of curve. Nine and one-half months after glycosuria was discovered the course of the blood glucose following food ingestion was followed during resting and working conditions. Both curves, Figure 7, show a normal fasting blood sugar, and although the postprandial peak is higher than those found in normal subjects, 192 mgm. per 100 cc., this tolerance test was made while the subject was still restricting her diet. The rapidity with which the blood sugar returns to the fasting level is not characteristic of diabetes mellitus. The urine remained sugar-free. The effect of work is marked and is similar to its effects on the curves of normal subjects.

*C. Obesity.* Case 23, Figure 8, has been approximately fifty per cent overweight for the past seven years. She was selected for these experiments because of characteristics of "endogenous"

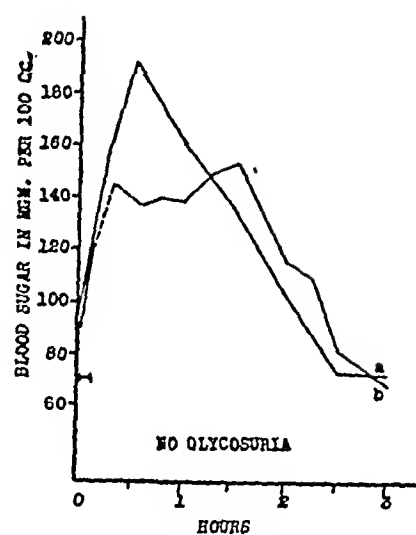


FIG. 7. CASE 22.

— Rest. --- Period of Work. Work in kilogrammeters. Curve (b) 4,274. |—| Glucose test meal 60 grams.

obesity.<sup>2</sup> Reduction has been persistently attempted but with little permanent success. At the time these tests were made the subject had been on a liberal unrestricted diet for several months.

The blood sugar findings following the ingestion of glucose are problematic, and will be taken up in the discussion.

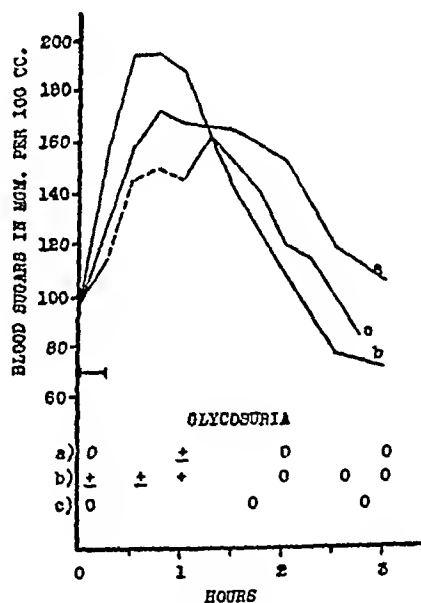


FIG. 8. CASE 23.

— Rest. --- Period of work. Work in kilogrammeters. Curve (c) 4,264. |—| Glucose test meal 57 grams.

<sup>2</sup> "Endogenous" obesity is used to denote those few patients resistant to weight loss by the usual methods. No other deductions as to the nature of the disturbance are inferred.

*D. Epilepsy on a ketogenic diet.* Case 24 is interesting not only from the standpoint of the effect of exercise on the blood sugar values, but also because of the excellent physical condition of this subject who has been on a ketogenic diet for nine years. These experiments on the blood sugars were carried out to determine the blood sugar characteristics under conditions of work and rest, of a patient with an extremely low carbohydrate intake (Figure 9). The three experiments, one rest curve and two curves with exercise, which resemble fasting blood sugar curves, were determined following the ingestion of the patient's usual breakfast containing seven grams of preformed carbohydrate and 15 grams of available glucose.

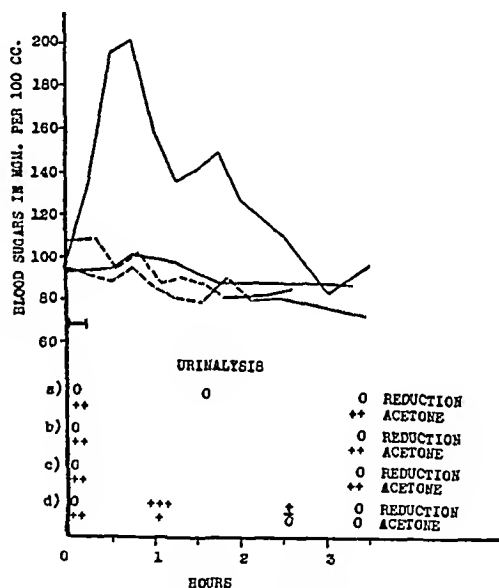


FIG. 9. CASE 24.

— Rest. --- Period of work. Work in kilogrammeters. Curve (b) 19,456. Curve (c) 25,254. |—| Mixed meal, Curves a, b, c. Carbohydrate 7 grams, protein 10 grams, fat 37 grams, calories 431, glucose equivalent 15 grams. |—| Glucose test meal 64 grams, Curve d.

#### Group V. Normal subjects

This group is composed of three adult males, one a physician and the other two students. Cases 25 and 26, the two students, ate mixed meals for all of the blood sugar determinations made, while the blood sugar studies on Case 27 were made following the ingestion of glucose. The subjects,

Cases 25 and 26, had been on known diets of carbohydrate 250 grams, protein 75 grams, and fat 100 grams for three days previous to the experiments.

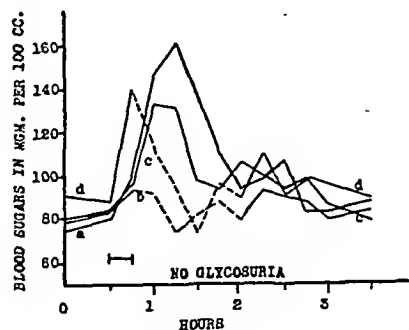


FIG. 10. CASE 25.

— Rest. --- Period of work. Work in kilogrammeters. Curve (b) 12,360. Curve (d) 13,423. |—| Mixed meal. Carbohydrate 98 grams, protein 22 grams, fat 33 grams, calories 777, glucose equivalent 112 grams.

The characteristics of the normal blood sugar curve need not be outlined here but the reader is referred to Case 25, Figure 10.

## DISCUSSION

Muscular exercise affects the postprandial blood sugar in the case of well controlled diabetes mellitus, with or without the use of insulin, in a manner similar to its effect on the non-diabetic individual. It is well known that mild types of diabetes mellitus are benefited by exercise. The cases of mild or well controlled diabetes presented in this paper differ from the normal subjects mainly in the greater elevation of the blood sugar after meals. Carbohydrate metabolism is regulated well enough to control the fasting blood sugar level.

Some difference in the general characteristics of the blood sugar curves is seen in the various cases grouped for discussion under mild diabetes mellitus treated without insulin (Cases 13, 14, 15, and 16), and well controlled cases under insulin management (Cases 1, 2, and 3). The three controlled cases on insulin therapy on the whole show less variation in the blood sugar levels than the four cases not taking insulin. The fasting values are, with the exception of Case 15, not abnormally high; and two of the four cases, Num-

bers 13 and 14 treated without insulin do not have high postprandial blood sugars. Two cases, Numbers 15 and 16, who showed glycosuria, were given diets at the time of the experiments to produce glycosuria, as has been mentioned, and do not on their usual regime have glycosuria. With the approximately normal blood sugars found in this group of well controlled diabetics it is not surprising that they should react to exercise as do normal subjects.

The effect of muscular exercise in the irregularly controlled diabetic individual appears to be more marked than in the well controlled case when judged by the difference between the level of the postprandial blood sugar during rest and immediately following exercise. The irregularly controlled cases of diabetes (Cases 4, 5, 6, and 7), dependent upon insulin, have a correspondingly greater variation in blood sugar levels. In all of the cases placed in this group the spontaneous adjustment of the blood sugar level during or after exercise, which seems to take place in the milder cases, is lacking. This is shown by the wide variation in the blood sugar levels at the end of the period of exercise when compared to the rest curve for the same time interval. As Lawrence (10) suggests the exercise seems to augment the effect of the insulin to a greater degree than would be expected merely from a summation of effects of insulin and exercise. Although the amount of work performed by the mild diabetics and the irregularly controlled severe diabetics may be comparable, the blood sugar levels vary widely. The body mechanism for regulation of the blood sugar seems in the case of the mild diabetics to be able to adjust itself to the effect of the exercise much better than it does in the non-diabetic individual. From an examination of the blood sugar curves, this regulating mechanism does not seem to exert its effect in the more severe cases of diabetes. This difference makes these cases more difficult to control without glycosuria or insulin shock, and especially so with a generous carbohydrate intake. The finding of a constant and marked effect from muscular work suggests that regular exercise performed at prescribed intervals might simplify the problem of insulin adjustment.

The third group, the uncontrolled cases of dia-

betes (Cases 8, 9, 10, 11, and 12), are probably uncontrolled because of improper spacing of the insulin dosage throughout the twenty-four hours. Because of the great changes in the blood sugar levels over rather short periods, these cases feel miserable much of the time, and even though hypoglycemia is absent, may complain of symptoms from the sudden shifts in blood sugar levels rather than the absolute values. In several of the subjects in this study, Cases 8, 10, and 11, particularly, we have been unable to differentiate between the symptoms resulting from true hypoglycemia and those resulting from a relative hypoglycemia following sudden changes in the blood sugar. Reactions which have taken place at blood sugar levels of 120 mgm. per 100 cc. are relieved promptly by administration of glucose in some form, as are those reactions which occur at blood sugar levels below 50 mgm. per 100 cc.

The effect of muscular exercise is difficult to determine because the drop in the blood sugar level from the insulin alone is such that the intake of food produces no postprandial rise in the blood sugar curve, and the amount of work that these patients were able to accomplish was small compared to the performances of the subjects in the other groups. Had these individuals been able to perform more work, the appearance of the resting blood sugar curves makes it improbable that the levels could be changed more rapidly by the aid of exercise than it was by insulin alone.

The fall in the blood sugar at rest is particularly noticeable because of the high fasting level, and is probably due to the intake of a large quantity of insulin within a short period of time. In well or even irregularly controlled cases the administration of equally large doses of insulin is not accompanied by this rapid fall in the blood sugar. The explanation of this may also lie in the fact that these latter cases have better control of this blood sugar regulating mechanism by reason of their having some more available endogenous insulin than the uncontrolled cases. They may be able, because of this endogenous insulin, to control to some degree the fluctuations of the blood sugar and thus to hold them more nearly within normal limits, but when exercise is added to the ordinary strain upon this mechanism, large fluctuations in the blood sugar result. In these

cases which are irregularly controlled there may be sufficient endogenous insulin together with the exogenous insulin to control blood sugar fluctuations unless some additional factor, such as strenuous exercise, is present to nullify the effects of the body regulation of carbohydrate metabolism. The recent work of Soskin et al. suggests, among other things, that liver dysfunction in the storage of glycogen may account for the marked fall in the blood sugar at rest since he has shown that a changing amount of insulin in the blood stream is not necessary for a normal glucose tolerance, but that a normal liver is necessary (22).

The effect of muscular exercise on the blood sugar of the cases of renal glycosuria, hypotension, glycosuria of pregnancy, and obesity is similar to that found in the normal individuals performing similar amounts of work. This is to be expected since there is no known metabolic disturbance in these conditions which should affect the blood sugar levels during rest and work.

The response to exercise in the case of obesity (Case 23, Figure 8) is similar to that of normal individuals; the other blood sugar changes are not typical in every respect of a non-diabetic. Curve *a* remains high for a longer time than is usual, while Curve *b* has an elevation higher than normal with resulting glycosuria. On the other hand, the return to the fasting level is rather prompt, and the curve with exercise shows some lowering of the blood sugar during work. Hard physical work, however, was extremely difficult for this subject and as a result the work accomplished was slight compared to that done by the other subjects in this study. One might expect, considering Wollmer's (4) findings that the blood sugar of obese subjects would react to exercise as does that of normal subjects.

The epileptic subject, Case 24, Figure 9, is introduced as an illustration of the effect of exercise on the blood sugar concentration of a non-diabetic subject on a low carbohydrate (ketogenic) food mixture. Practically no fluctuation was noted in the resting curve, in fact it appears to be the curve of a fasting person. It is not surprising that the small amount of glucose—fifteen grams—does not produce a noticeable rise, and that the effect of exercise on the blood sugar curve is negligible. Bergmark (23) has shown

that as little as six and a quarter grams of glucose produces a definite elevation of the blood sugar curve during rest; and work in this laboratory confirms these observations. Our findings are not contrary to the results of his experiments as the glucose in our diet was derived from a mixed meal of high fat content which may lower the rate of absorption of carbohydrate. The work performed by this subject when compared to the work performed by the other subjects in these experiments seems more than ample to produce a leveling of any blood sugar peak which might occur following the ingestion of a mixed meal. In this case as in that of other normal subjects it has been found difficult to lower the blood sugar by work to a point greatly below the fasting level. Only the most strenuous exercise, marathon running and the like, is able to lower the blood sugar in the normal individual much below the fasting level and to deplete the glycogen stores to a point that results in hypoglycemia (2). Though this case, Number 24, has been on a diet containing less than seventeen grams of carbohydrate for the past nine years, it is evident that his glycogen stores must be sufficiently great to prevent lowering of the blood sugar from work.

The results obtained in the three normal or healthy subjects examined were consistent in every case. It was possible to abolish the postprandial rise in the blood sugar by partaking of sufficient exercise following the meal. The blood sugar course of Case 25, Figure 10, at rest following a mixed meal, shows more variation, perhaps, than one would expect to find; but this is explained by noting that the subject had been indisposed and had eaten very little the day before Curve *a* was made. The maximum elevation of the other three curves compares favorably with those of Cases 26 who ate mixed meals. As in the other groups, when the blood sugar rises rapidly, the removal of glucose from the blood is more marked following exercise than that found in the curves without exercise.

#### SUMMARY AND CONCLUSIONS

1. This paper reports the observations made on the effect of muscular exercise on the blood sugar of twenty-seven subjects. Twelve had diabetes mellitus treated with insulin; four diabetes mellitus treated without insulin; three renal gly-



cosuria, one of which was on insulin therapy; two hypotension; and one each glycosuria of pregnancy, obesity, and epilepsy; while three were normal individuals.

2. Exercise of sufficient intensity and duration will lower the postprandial blood sugar in controlled diabetes mellitus treated with or without insulin, in renal glycosuria, in hypotension, in obesity and in normal subjects.

3. Exercise in the uncontrolled diabetic, in the amounts performed in these experiments, does not have a noticeable effect in lowering the postprandial blood sugar.

4. Exercise seems to have the greatest effect in lowering the postprandial blood sugar in the irregularly controlled diabetic, when measured by the difference in the resting and exercising blood sugar levels at the end of exercise, and by the resulting clinical symptoms at this time.

5. Exercise of the intensity performed in these experiments is not sufficient to lower the blood sugar below normal fasting levels in non-diabetics.

The authors wish to express their appreciation to Dr. Louis Leiter for his many helpful suggestions throughout the study.

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# CLINICAL STUDIES OF THE BLOOD VOLUME. I. CLINICAL APPLICATION OF A METHOD EMPLOYING THE AZO DYE "EVANS BLUE" AND THE SPECTROPHOTOMETER

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In the investigation of many clinical problems the determination of changes in the plasma and total blood volume occurring either rapidly or over long periods is of considerable interest. Since the introduction of the dye method by Keith, Rowntree and Geraghty in 1915 (1), several authors: Griesbach (2), Seyderhelm and Lampe (3), Heilmeyer (4), H. P. Smith, (5, 7), Graff and Clarke (6), Uhlenbruck and Leyendecker (8), Brockmann (9) and Sunderman (10) have emphasized the unreliability of the original method for clinical uses. In 1935 Gregersen, Gibson and Stead (11) summarized the errors inherent in the earlier methods and developed a method (12) employing the blue azo dye "T-1824" recommended by Dawson, Evans and Whipple (13) determining the dye concentration of samples with the spectrophotometer. In this paper we will describe a modification (14) of this method used by us for investigation of clinical problems and discuss the method in light of our experience therewith.

## METHODS

The dye solutions used are so opaque that the head of the plunger cannot be seen when the syringe is filled. Lines are engraved on the plunger of a 10 cc. glass tipped syringe near the head and on the barrel near the open end in such a way that when these lines are opposed, the head of the plunger is opposite the 10 cc. graduation on the barrel. Each syringe is calibrated by weight of distilled water contained when filled to the tip. Accurate delivery of dye in amounts from 3 cc. to 10 cc. is thus assured. Blood for serum is delivered into 6 cc. pyrex tubes, a light coating of thin liquid petrolatum being used to prevent adherence of the clot to the tube.

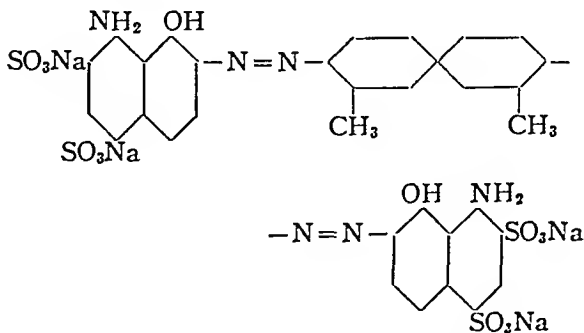
Hematocrit tubes are calibrated to contain 4 cc. and graduated into ten volumetrically equal

divisions numbered 1 to 10 from the bottom of the tube; these divisions are further graduated by linear measurement into 10 equal subdivisions. A 1.5 per cent aqueous solution of recrystallized potassium oxalate is used as an anticoagulant, the tubes being filled to the graduation numbered 2. Blood is run directly from the syringe into the tubes to the graduation numbered 10, and mixed with the oxalate by inversion.

All syringes used for the withdrawal of blood must be dry. Needles, syringes, etc., are sterilized by autoclaving.

## Preparation of dye solutions and determination of standard values

The same lot of T-1824 has been used throughout our investigation. This dye now called "Evans Blue," after Dr. H. M. Evans, was obtained from the Eastman Kodak Company. It is free from impurities, and is the same used by Dawson, Evans and Whipple (13) in 1920, as proved by its identical spectrophotometric absorption curve. It has the following structural formula:



Dye solutions are made up in freshly distilled water and filtered through a Jena sintered glass filter type 3-G-3. Neutral glass ampoules are

filled with 5 cc. or 10 cc. amounts, sealed and autoclaved. Solutions are not buffered. Prepared in this way no precipitation has been observed over periods of a year. A fresh ampoule is opened for each determination.

Standards are prepared in concentrations as shown in Table I. A fresh ampoule is opened for each standardization. A dye free serum blank for spectrophotometric reading is prepared by adding 0.2 cc. of normal saline to 1.8 cc. of clear serum.<sup>1</sup> An initial dilution in 0.85 per cent saline of the dye used for injection to one-tenth of the final dilution is made, and standards are prepared in triplicate by adding 0.2 cc. of the initial dilutions to 1.8 cc. of the same serum used in making the blank. The accepted value is taken as the average of the spectrophotometric readings of the three standards at wavelength of 6200  $\mu$  using cells 20 mm. in depth.

Table I shows the standard values obtained for 9 lots of dye so prepared. The variations encountered in individual standardizations, being about plus or minus 1.5 per cent, are within the limit of error of the technique for making up standards and of spectrophotometric reading. The optical properties of the dye are constant when prepared in the manner described. This procedure eliminates the preparation of a standard for each determination, the standard value for each lot of dye being used for the calculation of plasma volume in all determinations made with that lot.

#### PROCEDURE

All studies are carried out with patients under basal conditions. In many instances, blood volume determinations were accompanied by measurements of venous pressure by the direct method described by Evans (15) and blood velocity rates by the intravenous injection of "Decholin" as described by Winternitz, Deutsch and Brüll (16).

##### *Single volume determination*

A vein in the antecubital region<sup>2</sup> is punctured, and 7 cc. of blood is withdrawn for the dye free sample and a hematocrit. Stasis must be avoided. The dye is then injected

<sup>1</sup> For the 0.2 cc. amounts Van Slyke capillary pipettes delivering between the lines were used, and Ostwald pipettes made to deliver between lines for the 1.8 cc. amount. Stohl tips gave additional convenience.

<sup>2</sup> If venous pressure is measured before the volume determination the needle employed therefore may be used for this sample, and for dye injection.

TABLE I  
*Standard values of dye solutions*

Solution number	Concentration	Date of standardization	Dilution of dye in serum	$\Delta D$ at 6200 $\mu$ 20 mm. cells	Change from initial value
D	0.3% in H <sub>2</sub> O	September 18, 1935 September 24, 1935 October 10, 1935	1/500	1.0017 1.0135 1.0131	per cent +1.18 +1.13
G	0.3% in H <sub>2</sub> O	December 3, 1935 January 5, 1936	1/500	0.9898 0.9852	-0.47
K	0.3% in H <sub>2</sub> O	January 5, 1936 April 31, 1936	1/500	0.9805 0.9672	-1.36
F	0.15% in 0.85% saline	December 3, 1935 January 5, 1936 October 9, 1936	1/200	1.2335 1.2088 1.2336	-2.00 0.01
J	0.15% in 0.85% saline	January 5, 1936 March 24, 1936 October 9, 1936	1/200	1.2184 1.2170 1.2382	-0.12 +1.67
L	0.15% in 0.85% saline	March 24, 1936 April 31, 1936 October 9, 1936	1/200	1.2332 1.2126 1.2390	-1.67 +0.47
M	0.15% in 0.85% saline	June 8, 1936 June 28, 1936 July 20, 1936 August 3, 1936	1/200	1.2180 1.2204 1.2190 1.2274	+0.19 +0.10 +0.77
Q	0.1% in saline	November 10, 1936 December 18, 1936	1/100	0.822 0.820	-0.24
P	0.05% in saline	September 30, 1936 November 25, 1936	1/50	0.821 0.822	+0.12

through the same needle, in from thirty to forty seconds, and the syringe is rinsed with blood two or three times to insure delivery of all the dye. This needle is then removed from the vein and discarded.<sup>3</sup> An amount of dye approximating 0.002 mgm. per kgm. of body weight is given. A stop watch is started at the beginning of the dye injection, and subsequent samples are timed to the nearest ten seconds. Blood for serum samples and hematocrits are then taken at four or five minute intervals starting ten minutes after the beginning of dye injection, a fresh syringe being used for each sample. These samples can be taken through the same needle, clotting being prevented by injecting small amounts of saline between withdrawals. Dilution of samples with saline is prevented by drawing back blood into the saline delivery syringe before each sample is taken. Three or four hematocrits are obtained at regular intervals. A total of 30 to 50 cc. of blood are required for each determination. When sampling is

<sup>3</sup> Blood samples should never be taken through the needle used for the dye injection, as in our experience it is impossible to completely rinse all the dye from the hub or lumen of the needle even with repeated washing with blood or saline. All samples so withdrawn will have erroneously high concentrations of dye.

complete the blood velocity rate may be determined, the decholin being injected through the same needle used for sampling.

After clot retraction is complete, samples are centrifugalized for five or ten minutes at 2000 r.p.m. The serum is pipetted off and again centrifugalized to completely free it from cells.

Hematocrits are centrifugalized at 3000 r.p.m. for thirty minutes. Corpuscular volume is calculated by the formula:

$$\text{Per cent cells} = \frac{\text{Reading of packed cells}}{\text{Reading of fluid level} - 2.0}$$

The dye concentration of serum samples is determined with the Koenig-Martens spectrophotometer. Detailed discussion of the use of this instrument is beyond the scope of this paper; our procedure is as follows.

The absorption cells used are of fused quartz, made in matched pairs with optically ground faces,<sup>4</sup> 20 millimeters in depth. These cells can be emptied with a fine pipette so completely that rinsing between samples is unnecessary.

The spectrophotometer<sup>5</sup> is provided with a revolving photometer head on the circular scale of which the angular reading obtained when the image fields are matched is read. The initial setting with both cells when empty is such that the angular reading is forty-five degrees. The instrument is set to read at the wavelength 6200  $\mu$ , the point of maximum absorption of serum solutions of Evans Blue. One cell is then filled with the dye-free and the other with the dyed sample ten readings are taken and averaged, the position of the cells is reversed and ten more readings taken and averaged. The angular readings obtained before and after reversal of the cells will be less than or greater than forty-five degrees depending upon the position of the cell containing the most opaque (dyed) solution. The dye concentration of the samples is then calculated in terms of the net optical density ( $\Delta D$ ) according to the formula:

$$\Delta D = \log \cotangent \angle < 45^\circ + \log \tangent \angle > 45^\circ.$$

With the instrument properly aligned the logarithmic values of the two angular readings will be equal.

The  $\Delta D$  obtained for each sample is recorded and plotted on coordinate paper and the disappearance slope extrapolated to the ordinate as shown in Figure 1. The volumes are then calculated according to the following formulas:

$$\text{Plasma volume in cc.} = \frac{C \times \Delta D \text{ St.} \times D}{\Delta DU},$$

where  $C$  = the number of cc. of dye injected (taken as the calibration value of the syringe used),

<sup>4</sup> Made by Macalaster Bicknell Company, Cambridge, Mass.

<sup>5</sup> We have used the Koenig-Martens spectrophotometer made by Schmidt and Haensch, Berlin, Germany.

$D$  = the dilution of the dye solution used at which the standard value was determined,

$\Delta D \text{ St.}$  = the  $\Delta D$  of the standard of the dye solution used, as determined in cells of the same depth as used for obtaining  $\Delta DU$ ,

$\Delta DU$  = the  $\Delta D$  obtained by extrapolation of the slope of disappearance, as determined from the samples, to intersect the ordinate,

$$\text{Total blood volume in cc.} = \frac{\text{Plasma volume}}{100 - \text{per cent of cells}} \times 100.$$

The value used for the per cent of cells is the average of the values of all the hematocrits taken.

Red cell

volume in cc. = total blood volume—plasma volume.

Due to the method of reading the hematocrits to the top of the white cell layer, the red cell volume as here used includes the volume of the white cells.

#### *Repeated and continuous determinations*

For determining changes in plasma and total blood volume in the same individual, two types of procedure are available, termed by us the "direct" and "indirect" methods. The direct method consists of repeated single determinations in the same subjects at significant intervals, carried out as described above. The amount of dye used for each determination may be reduced if injections are to be made at short intervals.

In the indirect method, the slope of dye disappearance from the blood stream following a single injection is determined during a control period. The deviation in dye concentration of serum samples taken during an ensuing experimental period from the prolongation of the control disappearance slope represents changes in the plasma volume: an increase or decrease in dye concentration of a sample from that of a synchronous point on the disappearance slope indicates a reduction or increase in plasma volume respectively.

Changes in plasma and total blood volume occurring during the experimental period are calculated by the following formula:

$$\text{P.V.}_{1, 2, \text{ etc.}} = \frac{\text{P.V.} \times \Delta D \text{ sl.}}{\Delta D \text{ sp.}},$$

where  $\text{P.V.}_{1, 2, \text{ etc.}}$  = The plasma volume in cc. to be calculated from the  $\Delta D$  of serum samples taken during the experimental period,

$\text{P.V.}$  = The initial plasma volume in cc.,  
 $\Delta D_{\text{sl.}}$  = Dye concentration value of point on disappearance slope corresponding to serum sample taken during experimental period,

$\Delta D_{\text{sp.}}$  = Dye concentration of serum sample taken during experimental period,

ΔD

.8

.740

.7

(16)

MINUTES AFTER INJECTION

5 10 15 20 25 30 35 40

The method of extrapolation of the disappearance slope to obtain the optical density on which the calculation of plasma volume is based, and the portions of the curve designated by us "mixing curve," "disappearance slope," and "mixing time" are clearly shown.

The indirect method lends itself to the study of changes in plasma and total blood volume occurring over periods

of from a few minutes to several hours. When the experimental period is short (30 to 60 minutes), a blood volume is carried out in the usual manner except that blood samples for determination of the disappearance slope are taken over a period of time following the dye injection approximately equal to the length of the experimental period. When volume changes are to be followed over a period of several hours the initial volume determination is carried out 12 to 16 hours before the initiation of experimental procedures, from 15 to 30 mgm. of dye being injected. Patients are maintained fasting or on a light car-

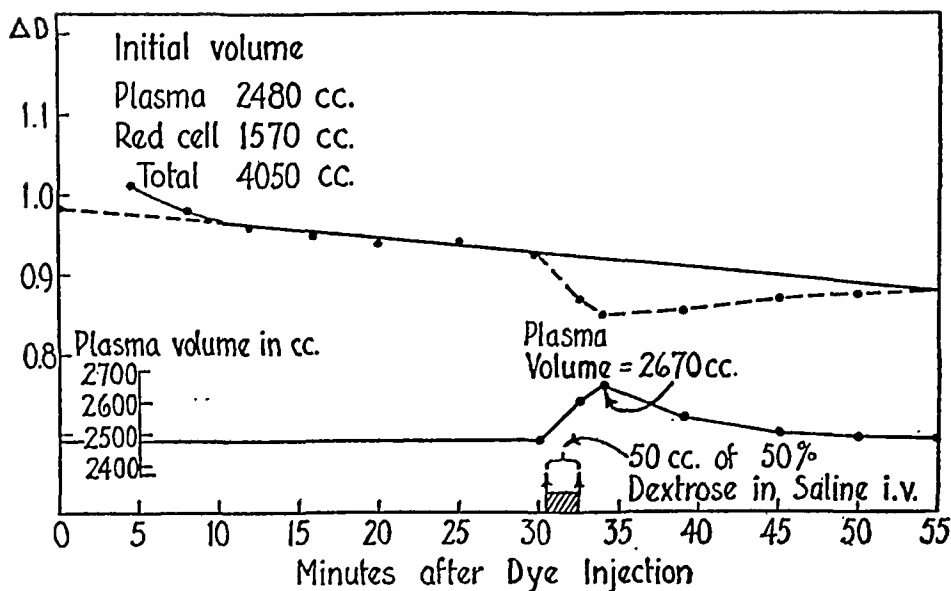


FIG. 2. CHANGES IN PLASMA VOLUME FOLLOWING RAPID INTRAVENOUS INJECTION OF 50 CC. OF 50 PER CENT DEXTROSE SOLUTION IN CASE 141, FEMALE, AGE 20 YEARS

The control disappearance slope, from the extrapolated value of which the initial volume is calculated and from the prolongation of which changes in volume are calculated is shown.

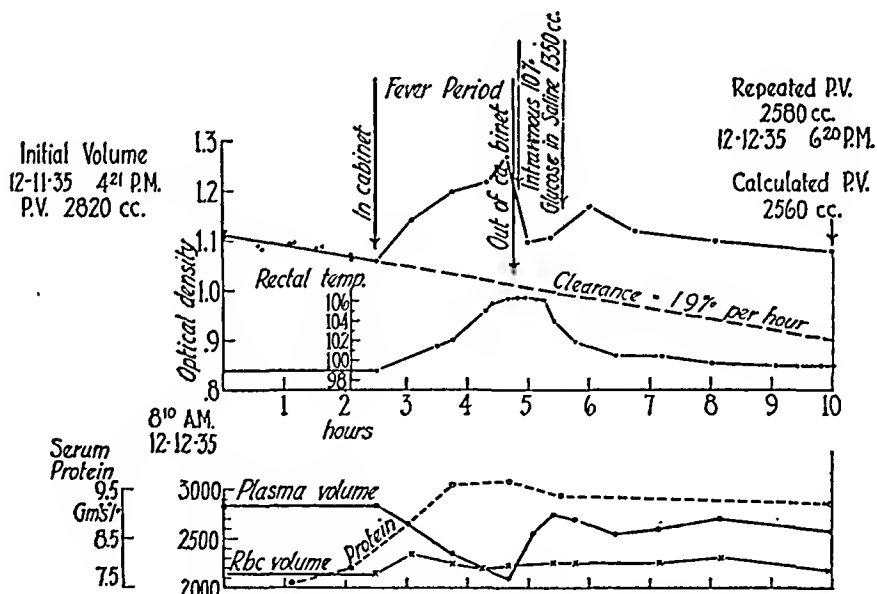


FIG. 3. CHANGES IN PLASMA AND RED CELL VOLUME DURING ARTIFICIAL FEVER IN A PARETIC, CASE 128

The initial volume was determined on the afternoon of the day preceding the experiment, about 16 hours before the zero hour on the chart. The control disappearance slope was determined over two hours and changes in plasma volume calculated from the prolongation of this slope and the initial plasma volume. The extreme concentration of blood during induction of fever with a rapid dilution following a large intravenous injection of 10 per cent dextrose in saline solution is shown, as well as the close agreement of final estimated and redetermined plasma volume at 10 hours. Red cell volumes were calculated from hematocrit and plasma volume values.

bohydrate diet during the entire observation period. Twelve to 16 hours after dye injection 5 or 6 blood samples for serum samples and hematocrits are taken at 20 to 30 minute intervals by separate venipuncture, for the determination of the control disappearance slope. The experimental procedure is then initiated and blood for serum samples and hematocrits is taken at significant intervals. At the end of the experiment, another volume determination is carried out, from 8 to 12 mgm. of dye being injected. Changes in plasma and total blood volume occurring during the experimental period are calculated by the formulae given above. The plasma volume estimated from the dye concentration of the sample taken just prior to the injection of dye for the final volume should equal the final plasma volume. The amount of blood withdrawn in this type of experiment varies from 100 to 200 cc. An experiment of this type is illustrated in Figure 3.

### CRITIQUE

#### *Factors affecting the accuracy of single determinations*

A complete discussion of the principles underlying the accuracy of this technique will appear elsewhere.<sup>6</sup> We are concerned in this paper only with the value of the method as applied by us to the clinical investigation of circulatory and other problems in humans.

#### *Colorimetry*

Estimation of the dye concentration of plasma or serum samples by means of the Dubosq colorimeter has been shown by Griesbach (2), Seyderhelm and Lampe (3) and Gregersen<sup>6</sup> to be unreliable. If the dye concentration of the standard is less than that of the unknown, the ratio between the readings of the standard and unknown is too low, and the calculated plasma volume falsely high; conversely, if the standard is more concentrated than the unknown, the calculated volume is falsely low. With the method herein described we have encountered clinically plasma volumes from 950 cc. to 6000 cc. In determining plasma volumes within this range with the Dubosq colorimeter, if the same amount of dye is injected and the samples are read against a standard of the same concentration in each case (as in the method employed by Keith, Rowntree and Geraghty (1); Hooper, et al. (17); Wollheim (18); and Goldbloom and Libin (19)) large errors will occur. In subjects with low plasma volumes the con-

centration of dye in the sample will be greater than that of the standard and an erroneously high volume value will be obtained, while the reverse condition will be encountered in subjects with large plasma volumes. Thus colorimetry with the Dubosq instrument fails to reveal extreme variations in plasma volume.

This type of error is eliminated with the spectrophotometer, since the absorption cells used are of equal depth, and the dispersion of light in both is equal. Both cells contain the same solvent (serum) in the same concentration, the color value thereof being cancelled; and with the instrument set at the wavelength of maximum absorption of Evans Blue in serum 6200  $\mu\mu$  (see Figure 4), the reading obtained accurately determines the difference in color between dye free and dyed samples due to the dye alone.

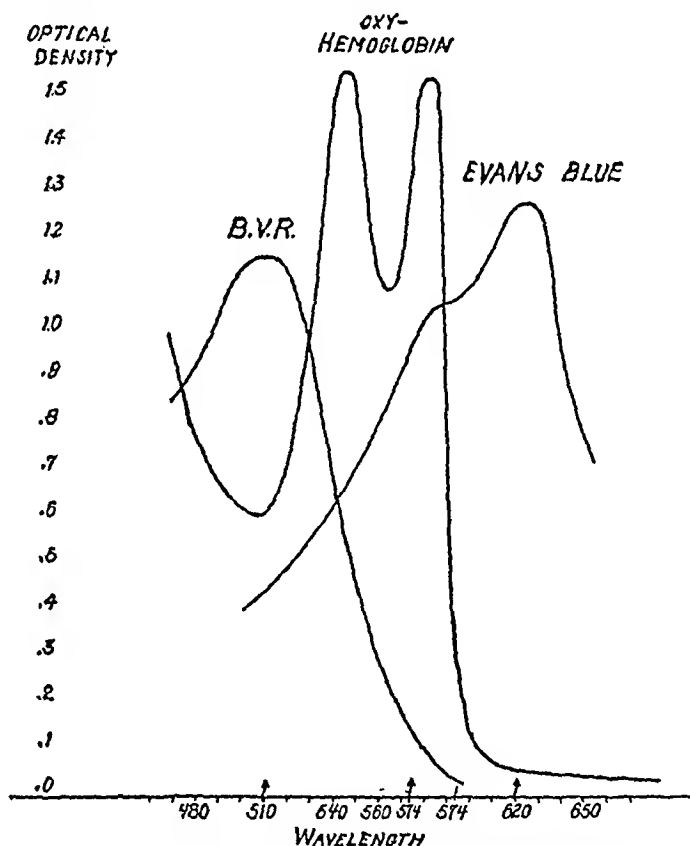


FIG. 4. SPECTROPHOTOMETRIC ABSORPTION CURVES OF OXYHEMOGLOBIN (HUMAN), BRILLIANT VITAL RED AND EVANS BLUE IN SOLUTION IN SERUM

These curves and those in Figure 5 were obtained with the Recording Spectrophotometer of the Color Measurements Laboratory of the Massachusetts Institute of Technology. The peak of the absorption curve of oxyhemoglobin occurs at the wavelength 576 mμ with this instrument, and at 574 mμ with the Koenig-Martens Spectrophotometers used by us.

<sup>6</sup> Gregersen, M. I. In preparation for publication.

No error due to residual dye is encountered when repeated determinations are made at short intervals, since here again both cells contain the same concentration of residual dye, and the readings obtained accurately measure the increase in color in the samples taken after the repeated dye injection due to that injection only.

In our experience, small amounts of lipemia do not produce inaccuracies as long as the degree of lipemia remains the same in all samples, but does result in uncorrectable errors if fat is disappearing from the blood stream during the sampling period.

#### *Hemolysis of samples*

Spectrophotometric absorption curves of Evans Blue and oxyhemoglobin (Figure 4) show that the absorption of hemoglobin at the wavelength 6200  $\mu\mu$  is little compared to its absorption at the wavelength 5740  $\mu\mu$ , the point of maximum absorption of oxyhemoglobin. Since both dye and hemoglobin in solution conform to the Beer-Lambert law, for any given degree of hemolysis of a serum sample stained with Evans Blue the per cent of error due to hemoglobin diminishes as the concentration of the dye increases. Thus if large amounts of the blue dye can be injected, as in animal experimentation, the error due to even considerable hemolysis is for practical purposes negligible.

Such large amounts of dye cannot be routinely employed in humans because of the undesirability of vital staining. With the amount of dye used by us,  $\Delta D$  values from about 0.4 to 1.2 are obtained and at these concentrations degrees of hemolysis as sometimes encountered even with careful withdrawal of blood may give rise to errors of from 2 to 5 per cent. It is therefore necessary in clinical investigation to have a method for correcting for hemoglobin.

Three possibilities obtain: hemolysis of dye free sample, dyed sample or of both samples. We have determined the ratio of the optical densities of oxyhemoglobin in serum solution at 5740  $\mu\mu$  and 6200  $\mu\mu$  to be approximately 40 to 1; and that of Evans Blue in serum at the corresponding wavelengths to be 0.8 to 1.<sup>7</sup>

<sup>7</sup> These studies were carried out with the recording spectrophotometer of the Color Measurement Laboratory of the Massachusetts Institute of Technology through the courtesy of Professor Arthur C. Hardy.

Based on the correction method of Graff and Clark (6) for oxyhemoglobin and brilliant vital red, correction for hemolysis of either sample is effected by the following formula:

$$D_2 = \frac{K_h A - B}{K_h - K_d},$$

where

$A$  = The  $\Delta D$  of the dyed sample read against the dye free sample at 6200  $\mu\mu$ ,

$B$  = The  $\Delta D$  of the dyed sample read against the dye free sample at 5740  $\mu\mu$ ,

$H_1$  and  $H_2$  are the  $\Delta D$  of oxyhemoglobin read against its solvent hemoglobin free serum at 5740  $\mu\mu$  and 6200  $\mu\mu$  respectively,

$D_1$  and  $D_2$  are the  $\Delta D$  of Evans Blue in hemoglobin free serum read against its solvent at 5740  $\mu\mu$  and 6200  $\mu\mu$  respectively,

$K_h$  = the ratio between the  $\Delta D$  of oxyhemoglobin in serum read against its solvent hemoglobin free serum at 5700  $\mu\mu$  and 6200  $\mu\mu$ ,

$K_d$  = the ratio of the  $\Delta D$  of Evans Blue in hemoglobin free serum read against its solvent at 5740  $\mu\mu$  and 6200  $\mu\mu$ .

The derivation of this formula is as follows:

$$(1) \quad A = D_2 + H_2, \quad \text{or} \quad H_2 = A - D_2.$$

$$(2) \quad B = D_1 + H_1, \quad \text{or} \quad H_1 = B - D_1.$$

$$(3) \quad \frac{H_1}{H_2} = K_h \quad \text{According to the Beer-Lambert Law constant for any colored solution; we have determined the value of this constant to be 40.0.}$$

$$(4) \quad \frac{D_1}{D_2} = K_d \quad \text{or} \quad D_1 = K_d \times D_2. \quad \text{We have determined the value of the constant } K_d \text{ to be 0.8.}$$

$$(5) \quad \text{Dividing (1) by (2),} \quad \frac{H_1}{H_2} = \frac{B - D_1}{A - D_2},$$

$$(6) \quad \text{Substituting (3) in (5),} \quad K_h = \frac{B - D_1}{A - D_2},$$

$$(7) \quad \text{Substituting (4) in (6),} \quad K_h = \frac{B - K_d \cdot D_2}{A - D_2};$$

$$\text{or} \quad K_h A - K_h D_2 = B - K_d D_2,$$

then

$$(8) \quad K_d \cdot D_2 - K_h \cdot D_2 = B - K_h A,$$

$$\text{or} \quad D_2(K_d - K_h) = B - K_h A,$$

and

$$(9) \quad D_2 = \frac{B - K_h A}{K_d - K_h}, \quad \text{or} \quad D_2 = \frac{K_h A - B}{K_h - K_d}.$$

This formula can only be applied when the dye free or at least one of the dyed samples is hemoglobin free. When the dye free sample contains no hemoglobin, correction of all hemolyzed dyed samples is simply made. When the dye free sample is hemolyzed, a non-hemolyzed dyed sample is read against it; the values thus ob-



tained for  $A$  and  $B$  are used in the formula and the corrected value for the dyed sample at  $6200\ \mu\mu$  calculated. The difference between the corrected value so obtained and the actual reading at  $6200\ \mu\mu$  constitutes the error at that wavelength due to the hemoglobin in the dye free sample.

Should some samples in the series also be hemolyzed, readings against the hemolyzed dye free sample are taken at  $6200\ \mu\mu$  and  $5740\ \mu\mu$  and the value determined for the error at  $6200\ \mu\mu$  due to hemolysis of the dye free sample added to the reading at  $6200\ \mu\mu$  and the corresponding value for this error at  $5740\ \mu\mu$  (forty times the amount at  $6200\ \mu\mu$ ) added to the reading obtained at  $5740\ \mu\mu$ , and these corrected values for  $A$  and  $B$  are used in the formula.

The dye free sample may be so greatly hemolyzed that the absorption at  $5740\ \mu\mu$  may be greater in the dye free than in the dyed sample in which case the value obtained for  $B$  must be used as an algebraically minus quantity.

#### *Mixing of dye in the blood stream*

Methods in common use are based on the assumption that injected dye is completely mixed in the blood stream in all cases in from three to six minutes and that there is no significant loss of dye from the plasma during the mixing period.

When samples are taken at three or four minute intervals over a period of thirty minutes after dye injection (see Figure 1), the dye concentration of successive samples falls rapidly at first, gradually reaching a constant rate of decrease. The initial rapid fall in concentration is due to mixing of dye in the blood stream, and this portion of the curve is termed by us the "mixing curve"; the subsequent portion in which the fall in concentration is constant, the "disappearance slope"; and the tangent point of these two curves the "mixing time."

Mixing time is related principally to the blood velocity rate as illustrated in Figure 6, in which it is seen that the mixing time of normals (as determined with 3 cc. to 10 cc. of dye) falls within narrow limits, averaging 7.5 minutes; that of cases of hyperthyroidism is more rapid; while in cases with velocity rates

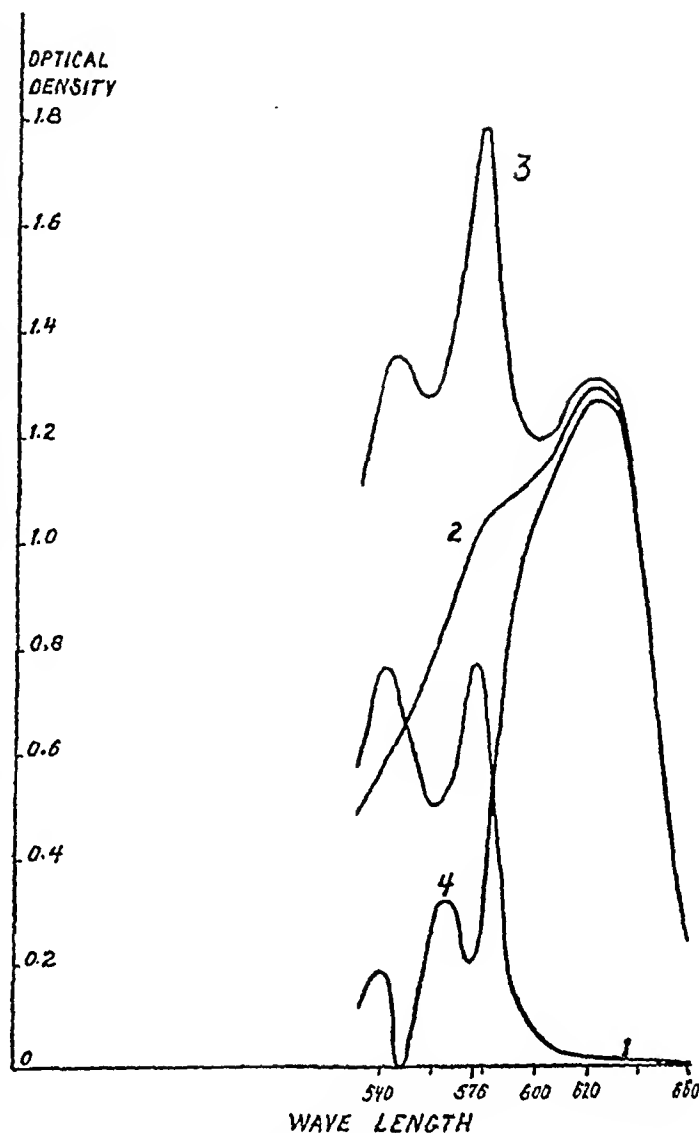


FIG. 5. SPECTROPHOTOMETRIC ABSORPTION CURVES OF HEMOLYZED SOLUTIONS OF EVANS BLUE

(1) Oxyhemoglobin in solution in hemoglobin free human serum read against its solvent. (2) Evans Blue in solution in hemoglobin free human serum read against its solvent. (3) Evans Blue in same concentration as (2) in solution in serum containing the same concentration of hemoglobin as (1) read against hemoglobin free human serum. (4) Solution (2) read against solution (1).

greater than twenty-five seconds, the normal average mixing time is exceeded; and that when the velocity rate is very slow the mixing time is greatly prolonged.

Figure 7 shows composite mixing curves in four groups of cases: normal, hyperthyroidism, moderate, and severe congestive heart failure. Analysis of these groups shows the degree of error to be encountered when the volume calculation is based on the dye concentration of a single sample taken three minutes after dye injection, as compared to the volume obtained by this

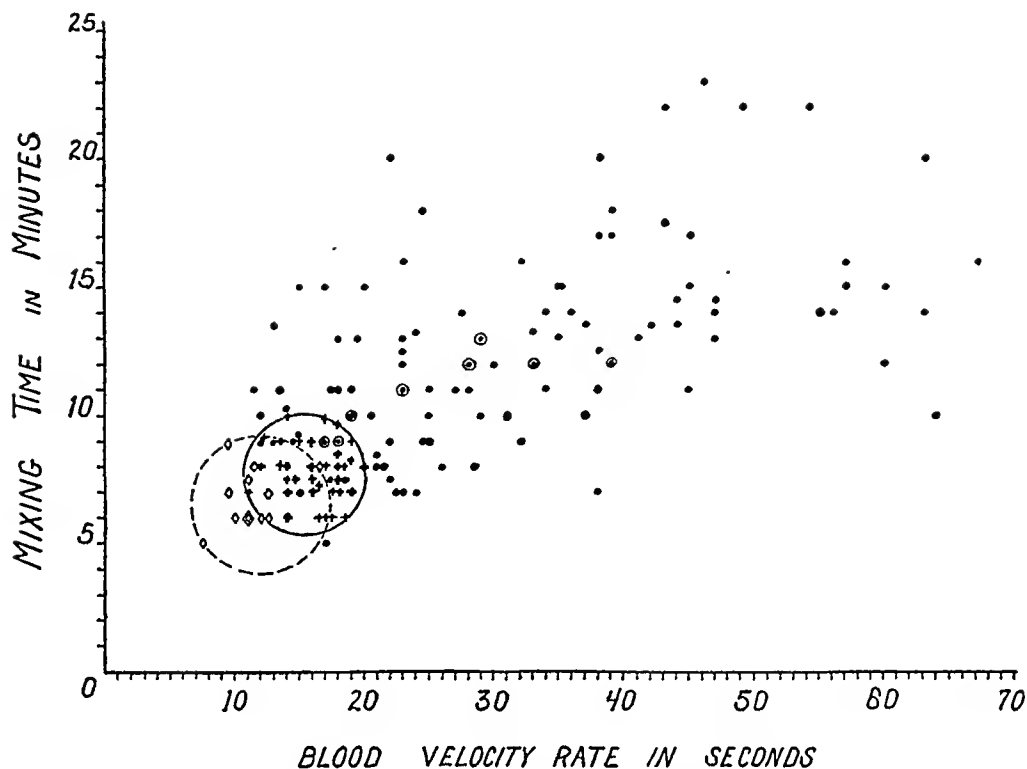


FIG. 6. THE RELATION OF MIXING TIME TO BLOOD VELOCITY RATE AS DETERMINED BY THE INTRAVENOUS INJECTION OF "DECHOLIN" IN 31 NORMAL PATIENTS, 11 WITH HYPERTHYROIDISM AND IN 60 DETERMINATIONS IN 36 PATIENTS IN VARYING STAGES OF CONGESTIVE HEART FAILURE

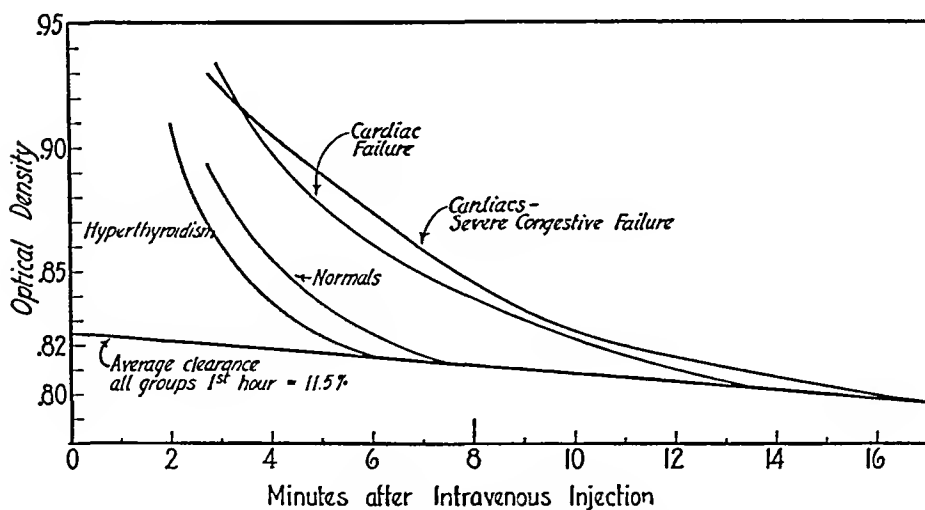


FIG. 7. COMPOSITE MIXING CURVES, REPRESENTING AVERAGE CURVES OF 5 PATIENTS WITH HYPERTHYROIDISM, 15 NORMALS, 9 WITH MODERATE AND 7 WITH SEVERE CONGESTIVE HEART FAILURE

method to be great. Since the dye is never completely mixed in three minutes, dye concentration of a sample taken at this time is always too high and the error is always in the direction of a falsely low volume. The average error in these four groups is 7.5 per cent for normals, 2.4 per cent for hyperthyroidism, 15.7 per cent for moderate and 19.6 per cent for severe congestive heart failure.

#### *Disappearance of dye from the blood stream*

Dye is removed from the blood stream principally by phagocytosis in the reticulo-endothelial system. It is not found in the urine of individuals with normal kidneys. We have been unable to demonstrate by spectrophotometric examination the presence of Evans Blue after intravenous injection in the edema, pleural or ascitic fluid of patients with congestive heart failure; in the ascitic fluid of patients with cirrhosis of the liver; in the edema, pleural or ascitic fluid of dogs rendered edematous by plasmapheresis; in the cerebrospinal fluid of a paretic patient; or in the fetal cord blood serum or amniotic fluid of a pregnant woman delivered by Caesarean section.

While we have not attempted to recover

Evans Blue from thoracic lymph, the observation of Smith (20) of the presence of brilliant vital red, a related colloidal dye, in thoracic lymph shortly after intravenous injection, indicates that dye may diffuse into lymph during the observation period. Plasma volume determinations based on the dye concentration of a single sample, even though taken when mixing is complete may accordingly be in error to the extent of this loss into the lymph.

In the method herein described the plasma volume calculation is based upon a value obtained by extrapolation of the disappearance slope to the time of injection. *This gives the dye concentration that would theoretically obtain were all the injected dye to be mixed with all the circulating blood before any blood has been withdrawn or any dye has disappeared from the blood stream.* This procedure minimizes errors due to diffusion of dye into lymph, and variations in initial rate of mixing in and disappearance from the blood stream.

#### *Hematocrit*

The dye method is essentially for the determination of the plasma volume, and the validity of the total blood volume calculation, based on hematocrits, rests on the assumption that hema-

TABLE II  
*Repeated basal plasma and total blood volume determinations in individuals over varying lengths of time*

Subject	Experiment number	Date		Age	Sex	Weight	Amount of Evans Blue given intravenously	Plasma volume		Total blood volume		Hematocrit
								cc.	cc. per kgm.	cc.	cc. per kgm.	
W. A.....	46A	June	23, 1935	28	M	71.8	18	2985	41.6	5600	78.1	46.7
W. A.....	46C	June	21, 1936	29	M	71.6	9	3170	44.3	5540	77.4	42.9
A. H.....	82A	September	18, 1935	30	M	62.7	10	2610	41.6	4620	73.5	43.4
A. H.....	82B	June	17, 1936	30	M	63.3	15	2530	39.5	4480	70.8	43.0
J. M.....	196A	January	8, 1936	28	M	73.3	30	2760	37.7	5380	73.5	48.6
J. M.....	196B	February	19, 1936	28	M	71.0	30	2630	37.0	5110	71.9	48.6
J. S.....	198A	March	11, 1936	43	M	62.8	30	2280	36.3	3950	62.7	42.2
J. S.....	198B	April	15, 1936	43	M	61.7	30	2320	37.7	3740	60.5	37.7
J. B.....	202A	May	22, 1936	44	M	68.7	30	3020	44.0	5400	78.6	43.0
J. B.....	202B	July	1, 1936	44	M	72.5	30	3030	41.8	5320	73.3	43.0
L. S.....	86A	October	2, 1935	23	M	59.6	12	2340	39.2	4180	70.2	44.1
L. S.....	86B	October	9, 1935	23	M	60.2	10	2360	39.3	4200	69.8	43.9
J. G. G...	1	November	7, 1934	37	M	72.6	10	3120	42.8	5530	76.3	43.8
J. G. G...	2	November	14, 1934	37	M	72.6	10	3010	41.5	5340	73.6	43.7
J. G. G...	32A	May	26, 1935	37	M	70.4	12	2945	41.8	5350	76.0	41.8
J. G. G...	132	December	19, 1935	38	M	69.5	15	3000	43.2	5280	76.1	43.2

toctrit values obtained under basal conditions from peripheral blood represent the true cell concentration of blood throughout the body. While there may be differences in corpuscular volume of central and peripheral blood, we feel that the differences observed in hematocrit values as determined by this method are of clinical significance.

### *Toxicity of Evans Blue*

Gibson and Gregersen (21) in 1935 made comparative studies of the toxicity of brilliant vital red and T-1824 (Evans Blue) when administered intravenously to growing male rats and concluded that both dyes were non-toxic in doses up to 20 mgm. per kgm. Our standard dose of 0.002 mgm. per kgm. is 1/80th of the upper limit of safe dosage, and about one-tenth the amount of brilliant vital red (100 mgm. to

150 mgm.) required by earlier methods. With this amount of dye no gross vital staining or immediate or late toxic or untoward effects have been encountered by us in over 300 patients, many of whom have been injected several times.

### *Factors affecting the accuracy of the direct and indirect methods*

The reliability of the direct method rests on the assumption that the blood volume in the basal state is constant. Findings in 7 normal individuals in whom volume studies under basal conditions were made over periods of from a few days to several months are summarized in Table II. While it is possible that the plasma and total blood volume varies somewhat from day to day, the constancy of the volumes obtained in these subjects under basal conditions leads us to believe that by the direct method changes in

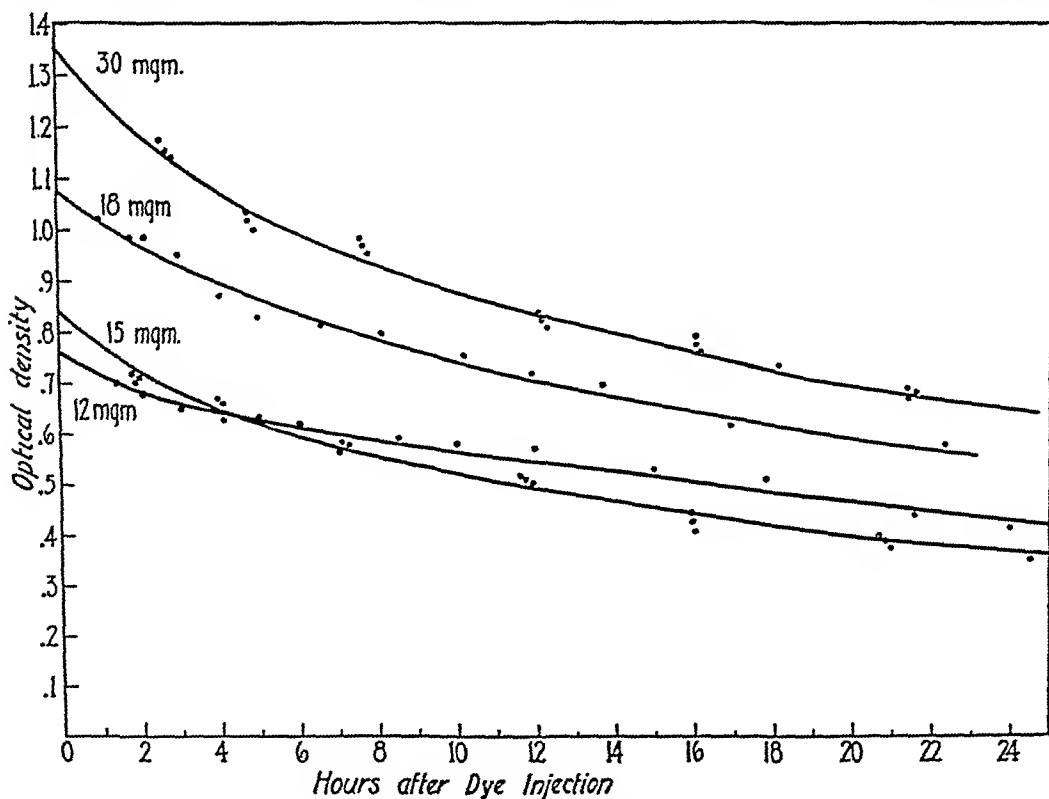


FIG. 8. DISAPPEARANCE FROM THE BLOOD STREAM OF VARYING AMOUNTS OF INTRAVENOUSLY INJECTED EVANS BLUE IN 4 NORMAL MALES

The rate of disappearance becomes practically constant from about 12 hours after injection on. In the curves of cases injected with 30 mgm. and 15 mgm. of dye the progressive fall in dye concentration of serum from serial samples taken at short intervals is shown. Disappearance slopes constructed from these points would be in excess of the prevailing disappearance slope for the 24-hour period.

excess of 5 per cent of the plasma volume can be reliably measured.

We have studied the disappearance from the blood stream of varying amounts of intravenously injected Evans Blue over 24 hour periods in four normal subjects. They were at complete bed rest and were maintained on a light carbohydrate diet and given water as desired throughout the observation period. An initial blood volume, with different amounts of dye in each case was carried out, and the disappearance of dye from the blood stream thereafter was followed by means of single or multiple blood samples taken at 1 to 3 hour intervals. Results are shown in Figure 8. Table III gives the rates

hours later samples for determination of the disappearance slope were taken by separate venipunctures at 20 minute intervals for two hours. The results of these studies are shown in Table IV. The average determined rate of disappearance during the 2-hour period, 16 hours after dye injection in these 10 individuals is 2.20 per cent per hour, which compares well with an average disappearance rate of 2.19 per cent per hour for the 16 to 20-hour period in the 24-hour group. (See Table III.) However, in some instances (Experiments 135 and 229, Table IV) the determined rate of disappearance is in excess of the average hourly rate of disappearance from the time of dye injection, a condition not in

TABLE III  
*Disappearance from the blood stream of intravenously injected Evans Blue in four normal males*

Experiment number	Date	Amount of Evans Blue given intravenously	Plasma volume		Blood drawn	Hourly disappearance rate (per cent)												Amount cleared in 24 hours	Initial $\Delta D$	Average disappearance at 16 hours	Disappeared at 16 hours
			Initial	Final		0	1	2	3	4	6	8	12	16	20						
						to 1	to 2	to 3	to 4	to 6	to 8	to 12	to 16	to 20	to 24						
		mgm.	cc.	cc.	cc.											per cent		per cent per hour	per cent		
203	May 27, 1936	30	3550	3160	220	6.07	5.61	5.04	4.70	4.11	3.25	2.69	2.26	2.06	1.89	52.5	1.365	2.75	44.1		
46A	June 23, 1935	18	2985	2720	180	5.63	5.28	3.82	3.33	3.12	2.73	2.66	2.49	2.14	1.83	49.8	1.083	2.54	40.7		
202	May 27, 1936	15	3020	2880	220	7.12	6.48	5.83	5.16	3.89	3.37	2.71	2.64	2.43	2.07	56.0	.829	2.92	46.8		
32A	May 26, 1935	12	2945	2680	185	5.13	4.72	3.50	2.72	2.18	2.12	1.90	2.16	2.11	2.08	45.0	.760	2.15	34.5		
Average												2.44	2.39	2.19	1.97	53.3		2.59	41.5		

of disappearance at various stages of the 24-hour period. Evans Blue leaves the blood stream very slowly and beginning 12 hours after injection the rate of disappearance may be considered constant for the purpose of the determination of control disappearance slopes.

The conformity of dye concentration of samples taken over a 24-hour period in these individuals to a smooth curve indicates that no considerable concentration or dilution of the blood occurs during the resting state. We are unable to attribute any of the slight variations in dye concentration of individual blood samples from the predominant curve to the ingestion of food or water, the elimination of waste products or to sleep.

We have studied the rate of disappearance during 2-hour periods 16 hours after dye injection in 10 normal individuals under basal conditions. For the initial volume determination, 24 to 30 mgm. of dye were used and 16

keeping with the finding of a constantly diminishing rate of disappearance over the 24-hour period. We believe these instances are evidence of a circulatory change induced by the taking of serial blood samples.

When blood samples are taken over a period of from thirty to forty minutes through the same needle or by repeated punctures a progressive decrease in the cell volume percentage occurs. Figure 9 shows the average per cent of decrease in hematocrit values taking place over a period of forty minutes, amounting to about 3 per cent, as observed in 105 patients. The rate of decrease is at first rapid, becoming less after about twenty-five minutes. A similar change in hematocrit values and a fall in dye concentration of serum was observed in serial blood samples taken in the 10 cases listed in Table IV, in general equal to the percentage of decline in hematocrit values. This decline in dye concentration was of such a degree that it could not

TABLE IV

*Disappearance from the blood stream of intravenously injected Evans Blue in 10 normal males 16 hours after injection*

Experiment number	Date	Amount of Evans Blue given intravenously	Initial plasma volume	Initial $\Delta D$	$\Delta D$ 16 hours	Elapsed time since injection	Disappearance since injection	Average disappearance from time of injection	Determined from disappearance slope
		mgm.	cc.				per cent	per cent per hour	per cent per hour
135	November 13, 1935	30	2970	1.703	1.101	15° 20'	35.3	2.31	2.48
128	December 12, 1935	30	2820	1.740	1.110	15° 48'	36.1	2.29	1.89
196	January 8, 1936	30	2760	1.805	.982	16° 19'	45.6	2.79	2.57
148	January 29, 1936	30	2380	2.060	1.293	16° 19'	37.1	2.28	2.42
200	April 1, 1936	24	2135	1.856	1.086	15° 27'	41.5	2.68	1.48
199	May 27, 1936	24	3230	1.263	.796	14° 42'	36.9	2.51	2.20
202	July 1, 1936	30	3030	1.600	.830	16° 10'	48.1	2.97	2.40
219	July 8, 1936	30	3040	1.625	1.039	17° 26'	35.7	2.07	1.83
225	July 22, 1936	30	2485	1.990	1.110	17° 26'	44.2	2.56	2.16
229	July 29, 1936	30	3260	1.520	.855	17° 28'	43.7	2.53	2.56
Average							40.43	2.50	2.20

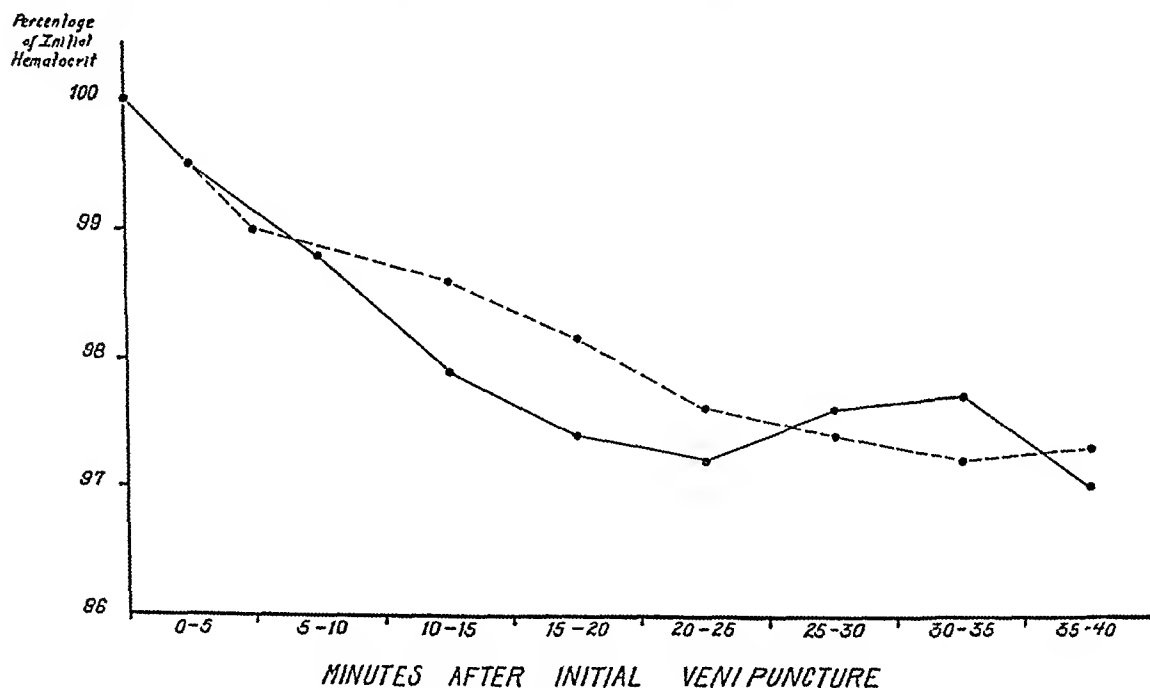


FIG. 9. DECLINE IN HEMATOCRIT VALUES OBSERVED ON REPEATED BLOOD SAMPLING

The solid line represents the average value of hematocrits, grouped in five minute periods, obtained during routine blood volume determinations in 60 patients with normal cardiovascular systems; the broken line the average values obtained in 55 decompensated cardiac patients. The initial hematocrit is taken as 100 per cent in each case, and all samples were taken without stasis, either through the same needle or by repeated puncture. The average fall in hematocrits, 3 per cent, is beyond the limit of error of the technique and greater than can be accounted for by blood withdrawn or the small amounts of saline (30 cc. in 40 minutes) injected to prevent clotting in the needle.

represent actual disappearance of the dye from the blood stream since it was in excess of the average rate of clearance prevailing during a corresponding period of the 24-hour curves shown above. We have observed the same phenomenon to occur in dogs (22). The effect of this decline in dye values tends to make the disappearance slope constructed from serial samples falsely steep. This is clearly illustrated in two of the subjects in whom dye clearance was followed for 24 hours. Several groups of serial samples were taken during the 24-hour period and in each instance, as seen in Figure 8, the slopes constructed from each group of samples are steeper than the corresponding portion of the 24-hour curve.

We do not believe these declines in hematocrit values and dye concentrations are due either to stasis, because of the precautions taken to avoid it; or to the injection of saline between withdrawals, since a similar condition obtains when a separate venipuncture is made for each sample. A sudden dilution of the circulating volume as a response to pain or hemorrhage would result in a decrease in both cell volume per cent and dye concentration but further studies indicate that the opposite condition, namely, a decrease in the circulating volume of both red cells and plasma, occurs. When repeated determinations are made in the same individual at short intervals, successive plasma volumes tend to differ from the initial volume, the difference being greater than can be accounted for by blood sampling. The percentage of change in plasma volume equals the percentage of change in the value of hematocrits taken during that volume determination. Results in three experiments illustrating this parallel in plasma volume change and hematocrit values are given in Table V. These findings are in keeping with the observations of Hemingway, Scott and Wright (23), who noted a similar drop in hemoglobin values and dye concentration of successive samples taken after injections of water blue in dogs; and of Chanutin, Smith and Mendel (24), who observed a progressive fall in hemoglobin values on repeated sampling.

This reaction is transient, and the volume of circulating plasma and red cells tends to return to the initial level, since the hematocrit and dye concentration values tend to rise towards the end

TABLE V  
*Changes in plasma, red cell and total blood volume and hematocrit values in repeated volume determinations in the same individual at short intervals*

Subject	Number of volume determination	Time between dye injections	Plasma volume	Change from initial value	Hematocrit	Change from initial value
			cc.	per cent		per cent
W.A.E.	1	0	3140		44.6	
	2	27 minutes	3000	-4.5	42.5	-4.6
	3	54 minutes	3065	-2.7	43.0	-3.6
W. C.	1	0	3660		45.9	
	2	27 minutes	3510	-4.1	43.9	-4.4
- W.	1	0	3170	0	41.4	
	2	27 minutes	3190	+0.6	41.4	0

of a series of samples. The effect of this reaction on the determination of disappearance slopes can be minimized by taking samples over a sufficient length of time. In our experience, in the long indirect method, control disappearance slopes should be based on at least five samples taken at 20 to 30 minute intervals. The optimal time after dye injection is from 12 to 20 hours, when changes in the hourly rate of disappearance are negligible and the dye concentration in the blood is still high enough for accurate spectrophotometric readings.

Changes in the pigment content of serum during the experimental period may affect the accuracy of indirectly calculated plasma volumes to some extent.

The average optical density at 6200  $\mu$  in cells of 20 mm. depth of dye free serum as determined in 20 normal men is about 0.1000. Since the amount of dye injected in the indirect method is such that optical densities in the neighborhood of 1.00 are obtained during the experimental period, an increase of 10 per cent in the optical density of the serum itself (due to increase in pigment content), would result in an increase of only 1 per cent in the  $\Delta D$  of the dyed serum read against the initial dye free sample. Errors arising from this source can best be avoided by maintaining subjects on fat free meals and are so slight as to be negligible.

We have made extensive studies by the indirect method of changes in plasma and total blood volume occurring during diuresis, fever, exercise and surgical operations. These conditions are accompanied by marked physiological dis-

turbances which might alter the rate of clearance from the blood stream. No method of quantitating such changes is available. However, since the experimental period selected is one during which the rate of dye disappearance is practically constant and very slow, changes in disappearance rate during an experiment will affect the accuracy of calculations of plasma volume based on the dye concentration of serum samples taken during the experiment, very little. In over 30 experiments of this type, plasma volumes calculated from the dye concentration of the sample taken just prior to the injection of dye for the repeated volume determination agreed within 5 per cent with the redetermined plasma volume.

#### SUMMARY AND CONCLUSIONS

(1) The application of a method for determining the plasma and total blood volume employing the blue dye Evans Blue and the spectrophotometer to the investigation of clinical problems is described.

(2) Colorimetric errors inherent in earlier methods due to turbidity of plasma, lipemia, residual dye in repeated determinations and hemolysis of samples are minimized by the use of the spectrophotometer, and a spectrophotometric method of correcting for hemolysis is described.

(3) Errors due to variations in dye mixing time occurring in different clinical states, and possible dilution of injected dye by lymph are eliminated by calculating the plasma volume from a value obtained by extrapolation of the slope of disappearance of the dye from the blood stream, as determined by multiple samples taken over a period of at least thirty minutes after dye injection, to the time of injection.

(4) By the "direct" method of repeated single determinations, volume changes of clinical significance in the same individual can be reliably measured at frequent intervals. By the "indirect method" changes in volume can be continuously followed for periods of from a few minutes to several hours.

(5) Certain factors affecting the accuracy of the indirect method are discussed. A physiological response to serial blood sampling consisting of a transient and variable decrease in

the circulating plasma and red cell volume renders accurate estimation of the rate of disappearance of dye from the blood stream difficult. Experimental procedures may alter the intrinsic color of the serum and rate of dye disappearance.

We wish to express our thanks to Professor Henry A. Christian for many helpful suggestions; to Professor Walter B. Cannon and Professor A. Baird Hastings for the use of spectrophotometers; and to Miss Evelyn Berstein for technical assistance.

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# CLINICAL STUDIES OF THE BLOOD VOLUME. II. THE RELATION OF PLASMA AND TOTAL BLOOD VOLUME TO VENOUS PRESSURE, BLOOD VELOCITY RATE, PHYSICAL MEASUREMENTS, AGE AND SEX IN NINETY NORMAL HUMANS

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Medical literature contains several works on the blood volume in normal persons, based on findings with the dye method of Keith, Rowntree and Geraghty (1) or modifications thereof, (2, 3, 4). Our justification for presenting an additional study is that the principal errors inherent in the earlier techniques are minimized by the dye method we have employed. By this method, described in detail in a previous communication (5), the absolute plasma volume can be measured with considerable accuracy, and the calculation of total blood volume is based on the plasma volume and the hematocrit values of venous blood samples. It also seemed worth while to consider the normal blood volume in relation to physical measurements of the individuals and to certain aspects of hemodynamics, to determine what interrelations exist.

This study comprises an analysis of the plasma and total blood volumes, venous pressures and blood velocity rates determined in 90 adult individuals, 49 of whom were males and 41 females. These subjects were hospital patients with no evidence by history or on physical examination of cardiovascular or renal disease, blood dyscrasias or debilitating disorders, and members of the hospital staff in good health.

Plasma and total blood volumes, venous pressures and blood velocity rates were determined with the subjects in a basal state. Results are summarized in Table I.

## Sex

The plasma and total blood volumes of all our subjects, considered by age and sex, are shown in Figure 1, and the corresponding plasma and total blood volume in cc. per kgm. of body weight in Figure 2. At once apparent is the striking

difference in volumes of males and females, the average absolute and per kgm. plasma volume of females being 22.5 per cent and 3.7 per cent less, and absolute and per kgm. total blood volume 28.8 per cent and 4.7 per cent less than that of males, respectively.

An extreme range of about 30 per cent above and below average plasma and total blood volumes is encountered in individuals, as shown in Figure 3, but no particular difference in range obtains between sexes. In about two-thirds of the cases in this series, plasma and total blood volumes were within 10 per cent above or below average values; of the remaining one-third, an approximately equal number were above and below that limit.

## Age

Analysis of absolute and relative volumes by decades (see Figures 1 and 2), shows a tendency in both sexes for values to remain at or rise somewhat above the average levels of the series during middle life and to decline with advancing age. The increase in total volume in terms of body weight during middle age is more pronounced in males than in females, while the decrease after the fourth decade, apparent in both sexes, is greater in females than in males, being about 16 per cent below middle age values in women and 8 per cent in men.

## Height, weight, and surface area

There is an increase in total blood volume with increasing individual height, weight and surface area as calculated from the nomogram of Boothby and Sandiford given by DuBois (6), the relationships being as shown in Figure 4. In relation to all three of these physical measurements, in about 70 per cent of our cases total volume is within 10

per cent above or below the average value for the group in both sexes. As regards weight only, those cases having volumes above these limits were predominantly muscular individuals, those falling below were for the most part tall and thin.

The relation of total volume in cc. per kgm. to variations in body weight is shown in Figure 5B, in which it will be noted that in both sexes, unit volume tends to rise with increase in weight of individuals, reaches a maximum at a lower weight level in females than in males, and thereafter

TABLE I

*The absolute and relative plasma and total blood volume, physical measurements, venous pressure, blood velocity rate and hematocrit in adult normal men and women*

Case number	Date	Age	Height	Weight	Surface area	Venous pressure	Blood velocity rate	Hematocrit	Plasma volume		Total blood volume	
									cc.	cc. per kgm.	cc.	cc. per kgm.
		years	cm.	kgm.	square meters	mm. H <sub>2</sub> O	seconds					
MALES—49												
3	November 23, 1934	26	178.0	70.4	1.88			44.6	2620	35.2	4720	67.1
6A	December 1, 1934	23	179.8	71.4	1.88			42.1	4045	56.8	6980	97.7
17	April 4, 1935	22		77.3		115	16	42.4	3890	50.3	6760	87.3
20B	November 29, 1935	26	186.3	74.6	1.97	60		44.4	2830	37.9	5090	68.3
32A	May 26, 1935	37	176.4	70.4	1.85	70	18	45.0	2945	41.8	5350	76.0
34	June 7, 1935	21	165.1	68.2	1.74	90	19½	45.3	3090	45.3	5650	82.7
46A	June 23, 1935	28	190.5	71.8	1.98	90	17	46.7	2985	41.6	5600	78.1
58	July 4, 1935	62	177.0	65.2	1.82	65	19	44.4	3140	47.0	5640	86.5
66	July 16, 1935	41	161.4	63.2	1.67	40	18½	44.5	2760	43.7	4970	78.6
69	July 19, 1935	19	178.0	65.0	1.80	70	14	45.4	2420	37.2	4360	67.0
73	July 22, 1935	26	187.0	66.0	1.88	115	18	48.7	2980	45.2	5800	88.0
74	July 23, 1935	24	171.4	76.2	1.88	115	17	47.8	2700	35.4	5160	75.3
82B	June 19, 1936	30	162.5	63.3	1.67	80	17	43.0	2530	39.5	4480	70.2
86A	October 2, 1935	23	166.3	59.6	1.65	80	14½	44.1	2340	39.2	4180	70.2
127	December 12, 1935	26	184.0	84.2	2.10	70	14	43.2	3200	38.2	5650	67.2
130	December 17, 1935	58		78.2		130	16	51.8	2400	32.0	5180	66.3
131	December 18, 1935	89		55.2		75	19	36.0	3210	58.2	5010	90.8
138	January 14, 1936	27	182.9	78.0	1.99	100	22	44.3	3190	40.9	5730	73.7
140B	January 15, 1936	22	175.2	76.1	1.91	80	20	44.7	3040	39.9	5500	72.2
142	January 18, 1936	43	173.0	71.0	1.83	120	17	48.8	2690	38.0	5260	74.0
148	January 31, 1936	39		54.3		110		45.2	2380	44.0	4350	80.1
155	March 17, 1936	22	176.0	79.4	1.94	45	16	49.4	3080	38.8	6080	76.7
161	March 24, 1936	27	174.5	72.4	1.85	120	15	48.3	2840	39.2	5490	75.8
177	May 6, 1936	60		68.0		95	17	49.3	2550	37.6	5030	74.0
180	May 13, 1936	19	153.4	48.6	1.43	120	14	48.4	1855	38.2	3590	73.8
193	June 4, 1936	58	170.0	64.6	1.74	30	18	43.3	2940	45.5	5190	80.4
196	January 8, 1936	28	161.3	73.3	1.77	100	17	48.6	2760	37.7	5380	73.5
197	February 26, 1936	38	180.2	63.3	1.79	85		40.2	3500	55.2	5850	92.4
198	March 11, 1936	43	157.4	62.8	1.63	85		42.2	2280	36.3	3950	62.7
199B	May 27, 1936	31	173.6	63.0	1.75	110	20	42.0	3230	51.3	5570	88.5
200	April 1, 1936	44	160.0	43.7	1.41	65		40.9	2135	48.8	3610	82.6
201	April 22, 1936	37	171.3	59.8	1.69	75	16	46.0	2860	47.8	5300	88.6
202	May 27, 1936	44	168.6	68.7	1.78	60		42.4	3020	44.0	5250	76.3
203	May 27, 1936	45	180.1	89.0	2.24	110		39.8	3550	39.9	5910	66.3
209	June 20, 1936	24	185.4	78.2	2.01		18	42.5	4000	51.3	6970	89.2
210	June 22, 1936	24	178.7	73.0	1.90	70	18	41.8	3560	48.7	6110	83.7
212	June 23, 1936	27	185.3	83.0	2.06	65	23	44.5	3660	44.1	6600	79.5
215A	June 27, 1936	25	180.1	69.5	1.87	80		41.4	3170	45.6	5410	77.7
219	July 8, 1936	57	173.2	62.5	1.73	85		38.1	3040	48.7	4920	78.7
223	July 16, 1936	41	185.3	75.0	1.97	65		48.8	2790	39.6	5450	72.7
224	July 21, 1936	30	167.6	59.0	1.66			48.0	2765	46.9	5325	90.3
225	July 22, 1936	51	170.0	65.5	1.73			42.6	2485	38.0	4330	66.2
226	July 23, 1936	38		73.7				43.6	3670	51.3	6510	90.8
227	July 27, 1936	39	172.6	76.6	1.89	60		46.0	2915	38.1	5400	70.6
229	July 29, 1936	44	172.1	78.1	1.91	65		46.9	3260	41.7	6140	68.7
231A	August 6, 1936	42	167.3	66.0	1.74			45.8	2845	43.2	5250	79.6
255	December 3, 1936	39	166.4	55.0	1.60	75		42.3	2530	46.3	4420	80.3
257	December 8, 1936	30	188.3	93.6	2.18			47.5	3620	38.7	6890	73.6
275	January 11, 1937	20	161.5	53.2	1.54		14½	46.2	2180	41.0	4050	76.2
Average		35.5	173.8	78.6	1.821	81.4	17.34	44.67	2948	43.08	5335	77.7

TABLE I—Continued

VOLUME. II

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TABLE I—Continued

Case number	Date	Age	Height	Weight	Surface area	Venous pressure	Blood velocity rate	Hemato-crit	Plasma volume		Total blood volume	
		years	cm.	kgm.	square meters	mm. H <sub>2</sub> O	seconds		cc.	cc. per kgm.	cc.	cc. per kgm.
FEMALES—41												
61	July 9, 1935	16	154.9	42.8								
63	July 11, 1935	26	168.0	55.6	1.37	55	16					
72	July 21, 1935	26	163.8	56.8	1.62	55	13½	37.3	1880	44.0	3110	72.6
83	September 19, 1935	28	163.7	48.2	1.60	60	14	43.4	2380	42.7	4190	75.3
120	December 15, 1935	31	167.7	68.6	1.50	80	14	37.9	2470	43.5	3970	70.0
122	December 6, 1935	41	149.8	48.8	1.77	90	14	41.0	2210	44.9	3750	76.2
136	January 14, 1936	28	166.3	61.5	1.42	90	14	39.7	2510	36.4	4150	60.4
141	January 16, 1936	22	170.0	57.0	1.68	95	17	39.5	1750	35.8	2990	59.3
143	January 20, 1936	55	176.3	49.0	1.63	60	17	37.6	2550	41.6	4100	66.7
145	January 23, 1936	42	155.0	80.8	1.43	45	18	38.7	2480	43.5	4050	71.0
149	February 17, 1936	27	167.4	70.0	1.79	95	18	41.7	1860	37.9	3190	65.1
152	March 13, 1936	20	160.0	56.5	1.78	110	16½	42.1	2170	26.9	3750	46.3
154	March 15, 1936	49	161.9	60.2	1.58	105	12	38.7	2360	33.7	3850	55.0
156	March 18, 1936	33	162.5	57.0	1.62	80	20	43.1	2120	37.9	3765	66.7
157	March 17, 1936	31	161.3	59.0	1.60	85	11	43.6	2360	39.2	4190	69.6
158	March 19, 1936	34	143.0	44.0	1.61	50	17½	41.2	2860	50.1	4870	85.4
160	March 21, 1936	27	167.4	53.4	1.31	50	17½	38.0	2850	48.3	4610	78.1
164	April 1, 1936	31	162.8	66.0	1.58	110	20½	38.3	2300	52.3	3730	84.7
168	April 10, 1936	26	152.5	45.0	1.71	100	13½	37.9	2405	45.1	3875	72.6
172	April 18, 1936	26		48.6	1.38	100	19	43.5	2390	36.2	4230	64.2
174	April 22, 1936	30	166.3	61.7		90	14	40.6	2045	45.3	3440	76.4
176	May 5, 1936	38	167.5	63.3	1.68	115	17	41.2	1925	39.7	3310	68.2
178	May 6, 1936	46	158.4	68.8	1.71	70	15	40.2	2820	47.3	4870	79.3
181	May 13, 1936	51	162.4	43.4	1.42	70	16	40.2	2490	39.9	4165	66.8
182	May 14, 1936	21	163.9	56.2	1.61	55	19	44.4	2300	33.4	4130	60.1
184	May 15, 1936	27	156.3	50.0	1.47	120	11½	42.6	1772	40.9	3090	71.3
186	May 21, 1936	37	152.2	58.0	1.54	80	18	41.5	2150	37.9	3640	64.7
206	June 10, 1936	52	62.2			95	17	38.5	1995	39.5	3240	64.8
207	June 16, 1936	21	162.4	59.2	1.62	70	24	38.2	2380	41.2	3860	66.6
208	June 16, 1936	23	160.0	47.0	1.47	65	13	43.0	2300	37.0	3435	55.3
211	June 23, 1936	19	167.4	54.0	1.59	65	15	37.8	2330	39.4	3750	63.4
213	June 25, 1936	36	184.1	54.0	1.71	95	17½	33.7	2500	52.1	3770	80.3
214	June 26, 1936	23	159.2	49.0	1.48	95	18	38.3	2550	47.2	4130	76.6
220	July 13, 1936	46	167.4	51.6	1.57	95	13	36.6	2820	52.2	4450	82.4
221	July 15, 1936	35	162.6	59.0	1.62	75	12½	36.9	2460	50.2	3995	79.5
222	July 16, 1936	37	161.3	49.6	1.50	95	16	43.8	2000	38.8	3570	69.3
247	November 17, 1936	48	165.1	63.8	1.69	60	16	36.3	2380	40.3	3730	63.2
258	December 11, 1936	23	161.3	46.3	1.45	75	14	38.7	2310	46.7	3770	76.1
262	December 23, 1936	21	156.3	46.0	1.40	95	11	42.5	2080	34.1	3620	56.7
266	September 17, 1936	20	157.0	43.4	1.39	50	15	39.7	2200	47.6	3650	78.9
272	January 8, 1937	20	151.8	47.4	1.40			39.1	1850	40.2	3030	66.0
Average		38.9	162.0	55.2	1.504	80.0	15.25	39.95	2284	41.5	3800	66.1

declines with further increase in weight, the decrease being more pronounced in women than in men. In relation to variations in height (Figure 5A) unit volume rises with increasing individual height, reaches a maximum value and remains quite constant with increases in height thereafter in both sexes. The composite relation of total volume to variations in both height and weight, namely, surface area (Figure 5C), clearly indicates the chief differences between the sexes in the relationship of volume to physical measurements; total blood volume in terms of surface

area is about the same in small men and women, but is considerably greater in large men than in large women. In addition, with increasing individual size, unit volume in males increases steadily at a gradually lessening rate, while in females it rises to a maximum well below the level of the male maximum value and declines rather sharply thereafter.

#### *Venous pressure and blood velocity rate*

We have observed no significant variations in either venous pressure or blood velocity rate with

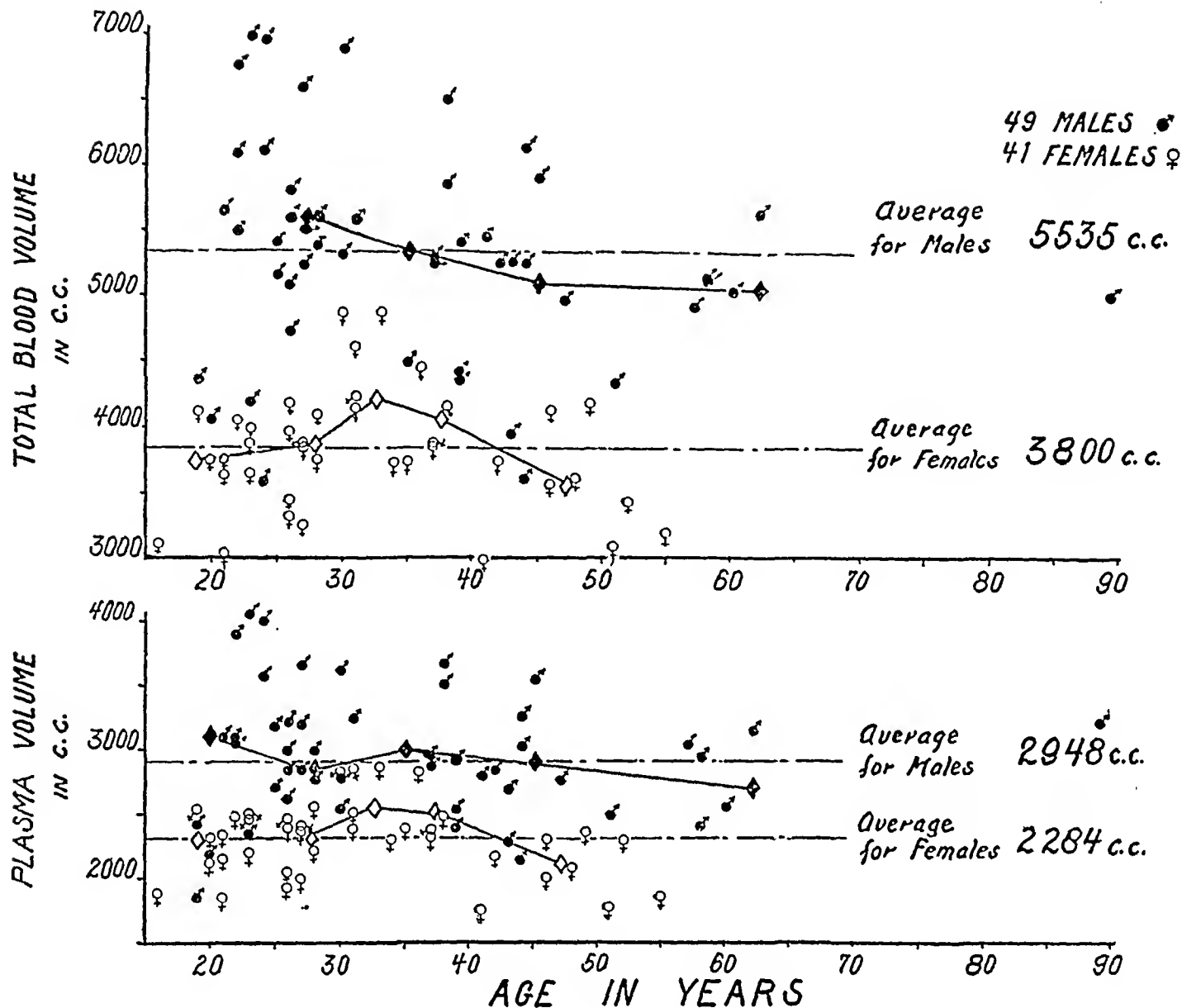


FIG. 1. PLASMA AND TOTAL BLOOD VOLUME IN 90 NORMAL MEN AND WOMEN WITH REFERENCE TO AGE

Both plasma and total blood volume of males is greater than that of females, and tend to diminish with advancing age in both sexes.

age in either sex (Figure 6). The degree of variation in individuals above and below average values for the whole group is less in the case of the blood velocity rate than in venous pressure in both sexes, but in both instances the extremes encountered in males and females are about the same. The average value of both determinations is appreciably lower in females than in males. The relation of total blood volume to variations in venous pressure and blood velocity rate is as shown in Figure 7. It is apparent that no interrelation between variations in these two measurements and blood volume exists in normal persons.

#### *Red cell volume*

In the method employed the red cell volume is based upon the hematocrit values of venous blood samples, being considered as the difference between total blood and plasma volumes. In this series the male hematocrit value ranged from 36.0 to 51.8 with an average value of 44.6; the female hematocrit from 33.7 to 43.8 with an average value of 39.9. Thus the average hematocrit value for females is 10.5 per cent less than that of males.

The computed average red cell volume of males is 2387 cc. and that of females 1514 cc., or 36.5 per cent less than that of males.

## CRITIQUE

Our findings are at variance with those in the literature. Thus Keith, Rowntree and Geraghty (1), Rowntree and Brown (7), Kaltreider, Hurtado and Brooks (8) and Silbert et al. (9) reported higher average absolute and relative total blood volumes for males than the average values of this series, and Bock (10) and Seyderhelm

Some of the wide divergence in values found by the above authors may be due to the limited number of cases studied, since the extreme range encountered in this series, comprising more cases than were included in any of those series referred to above, is plus or minus 30 per cent from average values. Pitfalls of the earlier methods described by Gregersen, Gibson and Stead (16)

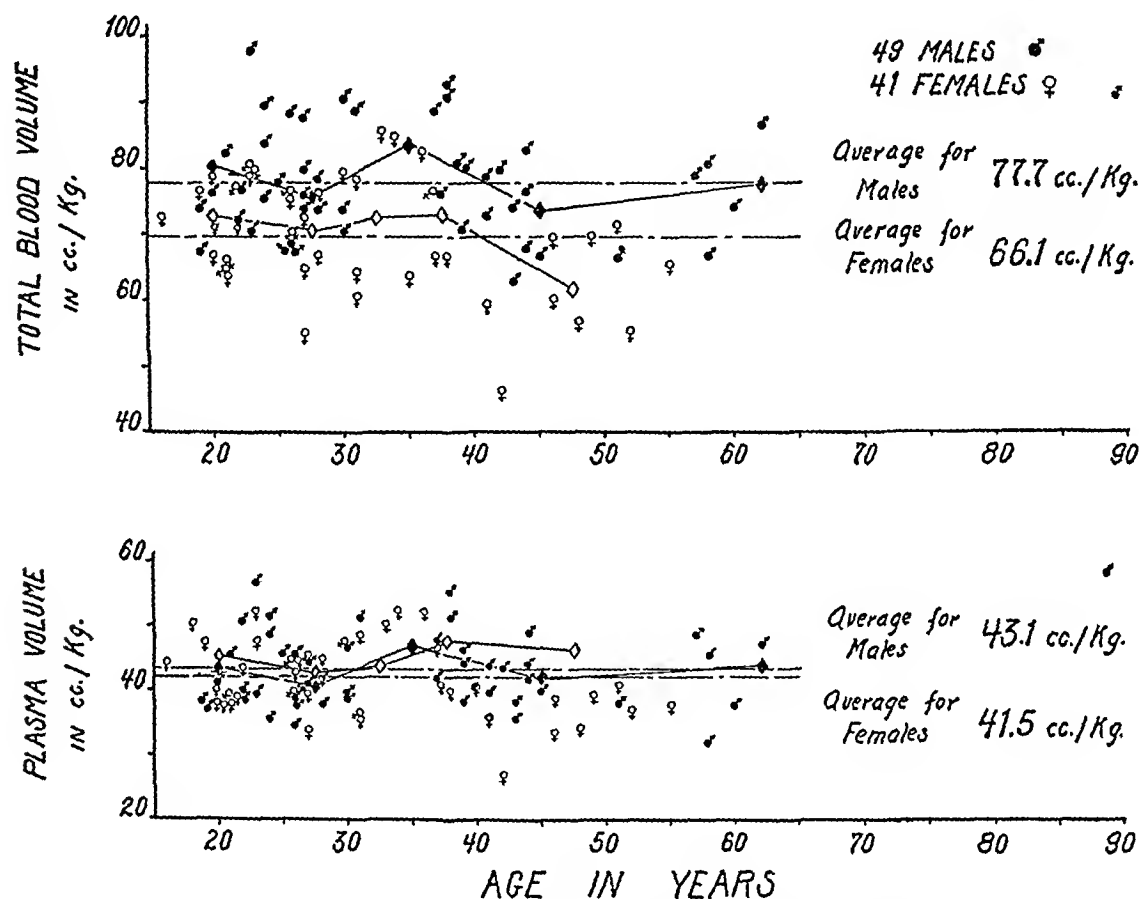


FIG. 2. PLASMA AND TOTAL BLOOD VOLUME IN TERMS OF BODY WEIGHT

The difference in total volume of males and females is due principally to the larger red cell volume of the male, the unit plasma volume of both sexes being nearly equal.

and Lampe (11) obtained higher relative total blood volumes. Wollheim (12) obtained a higher relative and lower absolute average total volume than our values. Kaboth (13) and Sparks and Haden (14) published values for women lower than those herein reported both as to absolute and relative total volume, while those of Uhlenbruck and Leyendecker (15) and Rowntree and Brown (6) were considerably higher.

and in a previous communication by us (5), account in large measure for this divergence. Thus, if the volume calculation is based on the dye concentration of a single blood sample taken before the dye is completely mixed in the blood stream, falsely low values will be obtained. Inequality of dye concentration between standard and unknown may produce large errors in either direction when the Dubosq colorimeter is used, as may

also errors due to hemolysis of dye free or dyed samples or both. While some of these errors may be compensating, it seems evident that values for normal blood volume as determined by the methods employed by the above authors cannot be regarded as reliable.

tissue as muscle and viscera and blood poor tissue as bone and fat. It is accordingly consistent that muscular persons should have relatively more and fat persons relatively less blood per unit of body weight than persons of more normal habitus and proportion of brawn and blubber. Thus Cases

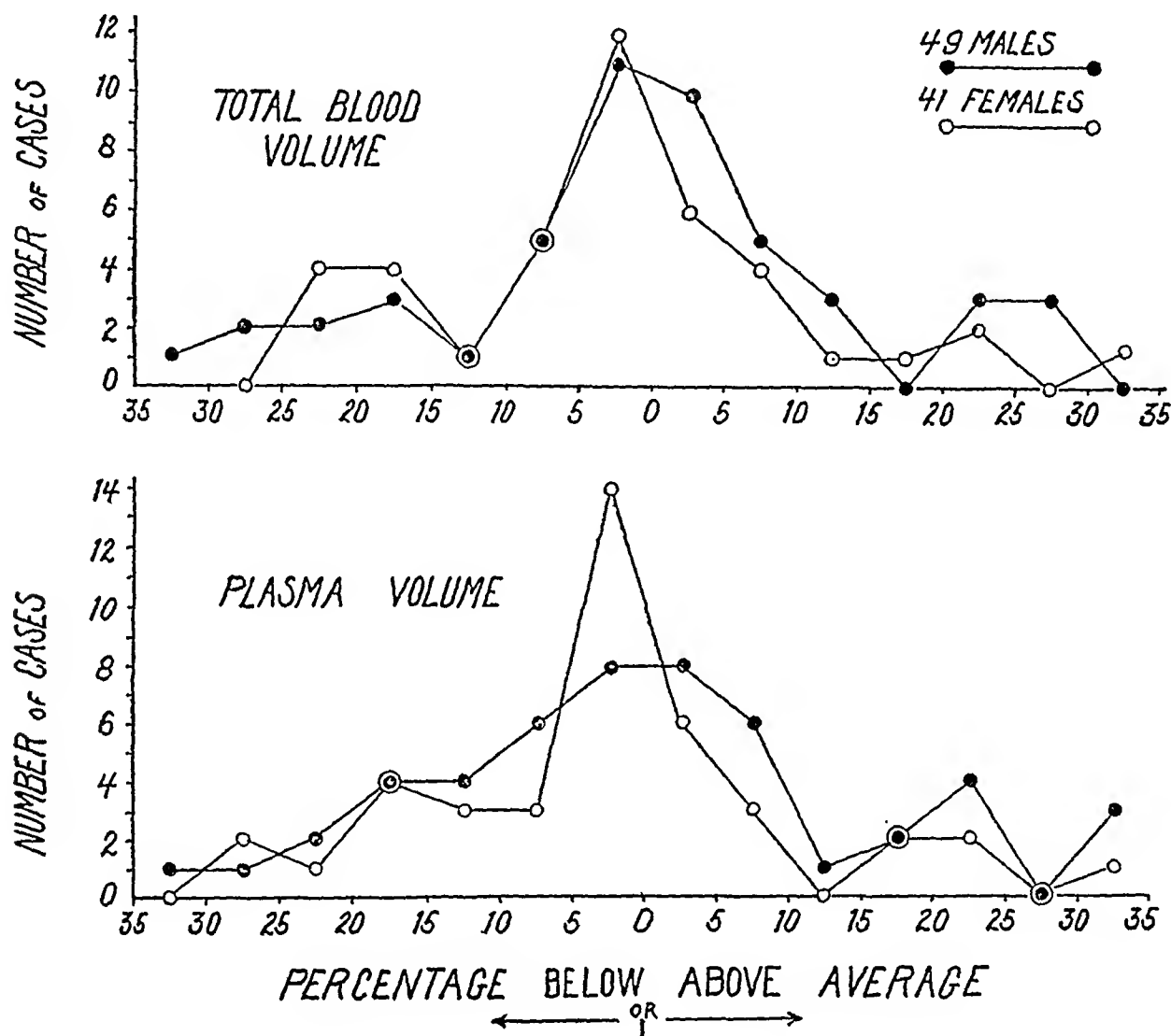


FIG. 3. DISTRIBUTION OF NUMBER OF CASES ABOVE AND BELOW AVERAGE VALUES FOR PLASMA AND TOTAL BLOOD VOLUME

About two-thirds of the cases are within 10 per cent above or below average values in both sexes.

Our analysis of the blood volumes of normal individuals, as determined by the method we have employed indicates that differences in plasma and total blood volume of individuals of comparable age, sex, height and weight may be considerable. That extreme variations in the amount of blood in the bodies of different persons exist is not incompatible with known individual differences in build, and varying proportions of such blood rich

6A, 156, 174, 209 and 224 are muscular persons leading physically active lives, and in all of these, both absolute and total volume per kgm. of body weight exceeds average values. Cases 140B, 145, 149, and 203, all obese persons, have absolute volumes equal to or above the average values, but less than the average value per unit of body weight. Cases 158, 208, and 226, all underweight, have absolute volumes slightly under, but

relative volumes much higher, than average values.

The greater blood volume of males than females is due chiefly to the higher red cell volume of the male, the plasma volume of the two sexes differing only slightly (Figure 1). This fact is in keeping with known observations of higher red

cell counts, hemoglobin and hematocrit values, basal metabolic rates (DuBois (6)), and vital capacities (Hutchinson (17) and Pratt (18)) of men as compared to women. It is of interest, however, that the total red cell volume of women as compared to that of men is considerably less

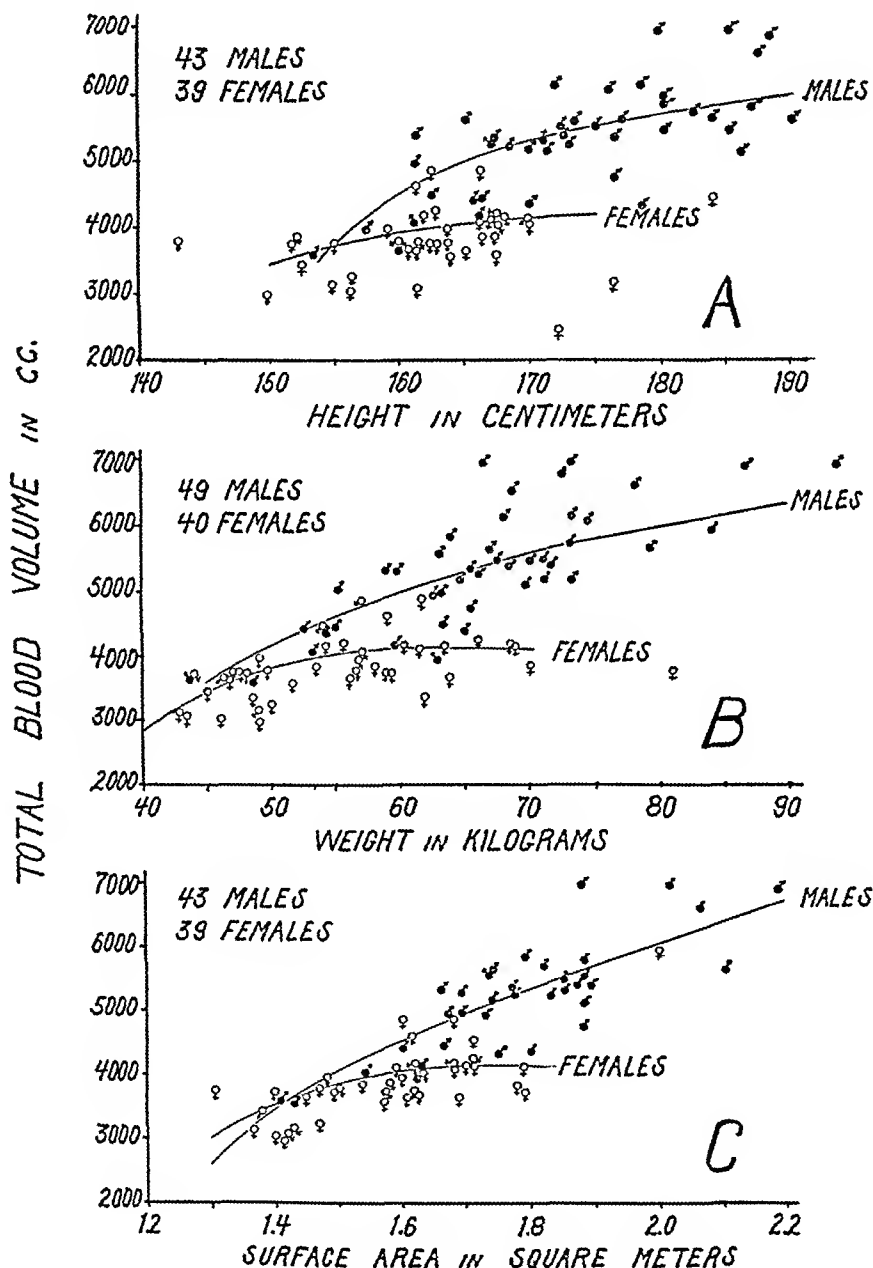


FIG. 4. RELATIONSHIP OF TOTAL BLOOD VOLUME TO HEIGHT, WEIGHT AND SURFACE AREA

With increase in size total volume in relation to surface area rises steadily in males, but tends towards a constant value in females.



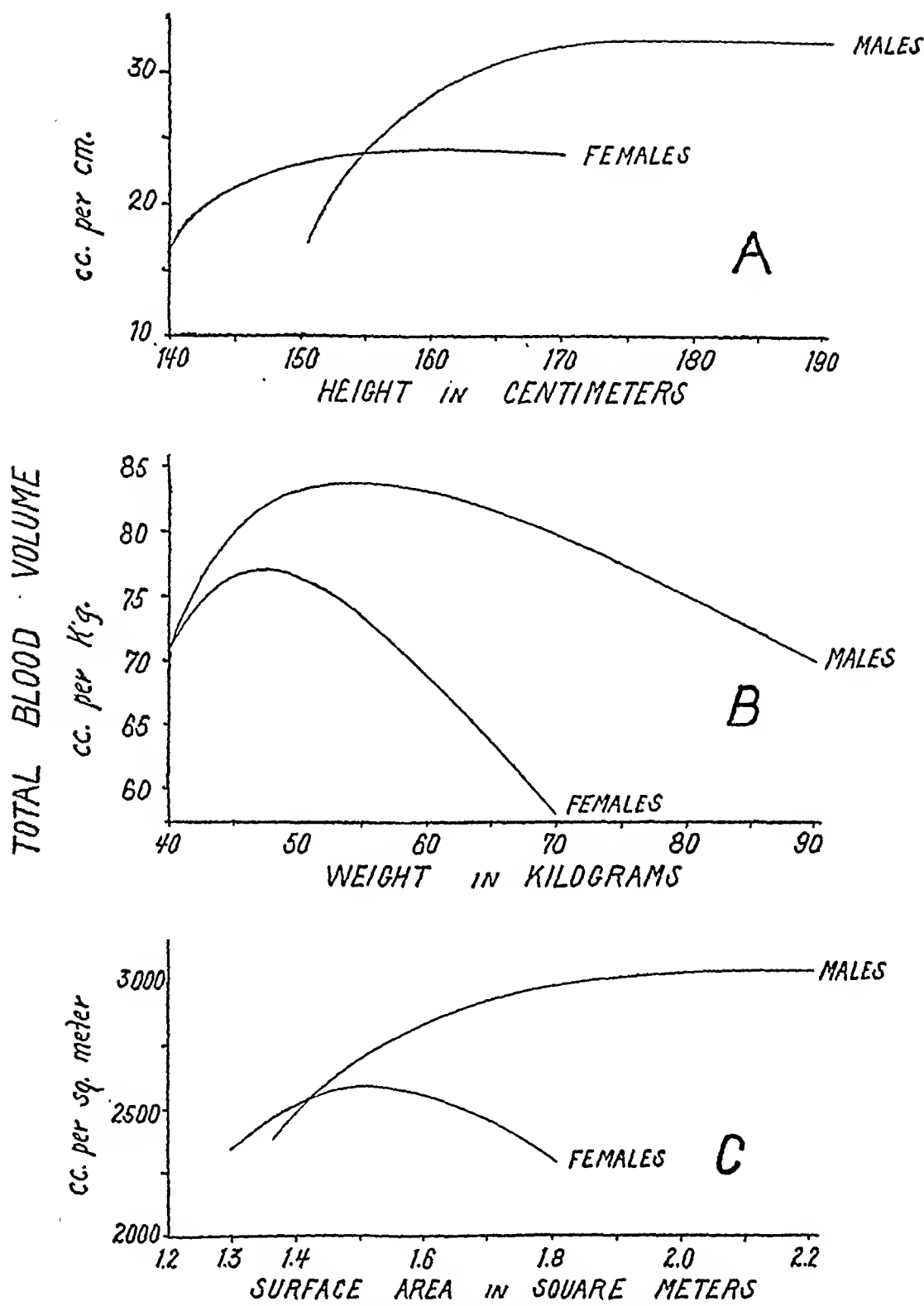


FIG. 5. RELATIONSHIP OF TOTAL BLOOD VOLUME IN UNIT TERMS TO DIFFERENCES IN INDIVIDUAL HEIGHT, WEIGHT AND SURFACE AREA

The diminution in unit volume with increasing weight is more pronounced in women than men. With increasing surface area, unit volume rises and tends toward a constant value in men, but decreases in large women.

than the percentage differences between normal values for red cell counts, hematocrits and hemoglobins would indicate. Thus while average hematocrit values in this series for women are only 10.5 per cent less than for men, the total red cell volume of women is 42.8 per cent less than that of men; the reason being the lower value of both the plasma volume and hematocrit of women. In this connection it should be pointed out that differences in absolute red cell volume of individuals

or of changes therein in the same individual cannot be determined on the basis of red cell counts or hematocrits alone. In each case, the plasma volume must be taken into consideration.

That there should be a considerable difference in the blood volume of men and women is not surprising when considered in terms of body weight, and the fact that men as a rule are more muscular and are apt to lead more physically active lives than women. In this connection cer-

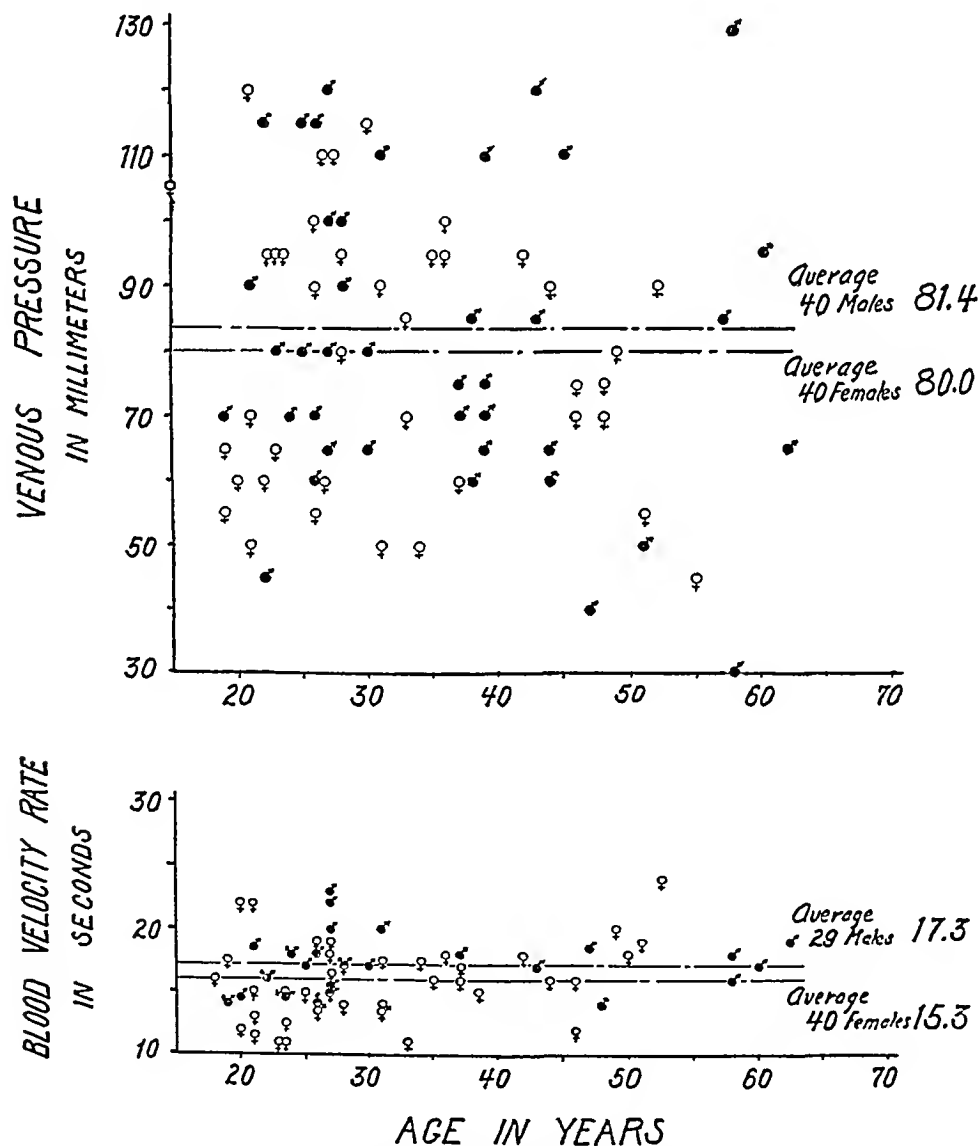


FIG. 6. VENOUS PRESSURES AND BLOOD VELOCITY RATES IN 90 NORMAL MEN AND WOMEN  
No relationship of these determinations to age exists in either sex.

tain cases in this series are of interest as exceptions that prove the rule. Thus Cases 156 and 174, both athletic women, have high relative volumes well within the male range, while Cases 82B and 127, men of poor muscular development

during the life span. Thus Krogh's modification of the Sage standards of basal metabolism (6, p. 157) shows a decrease of 7 per cent in calories per hour between the ages of 25 and 55. A decrease of about 17 per cent in vital capacity dur-

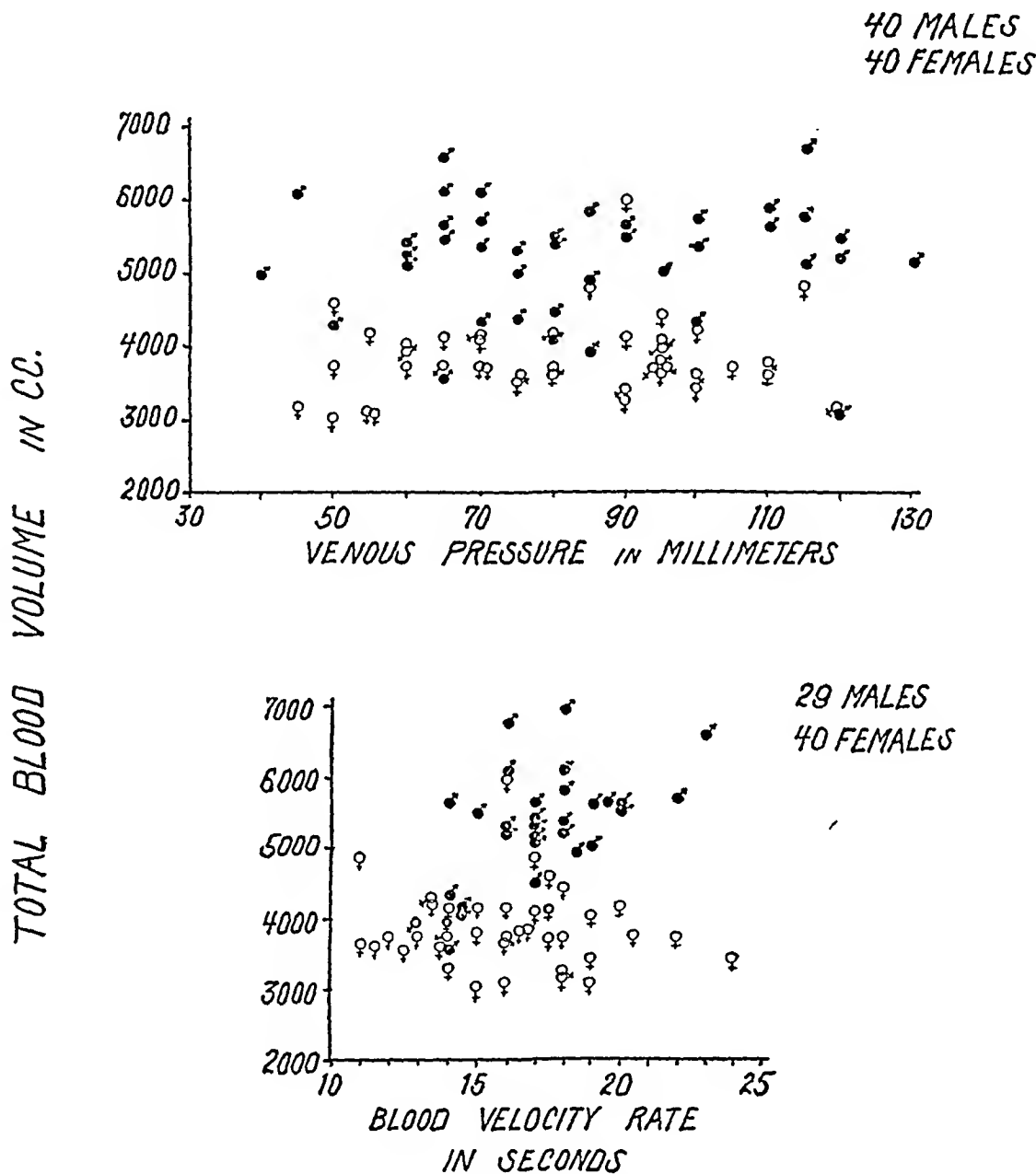


FIG. 7. RELATIONSHIP OF TOTAL BLOOD VOLUME TO VENOUS PRESSURE AND BLOOD VELOCITY RATE IN 90 NORMAL MEN AND WOMEN

No relationship to variations in these factors exists in either sex.

and sedentary habits, have relative volumes well below the male average.

The decline in total blood volume with increasing years, amounting to about 14 per cent in males and 10 per cent in females between the ages of 25 and 55 is in keeping with observations on changes in basal metabolism and in vital capacity

ing a similar age period is revealed in the studies of Hutchinson (17) and Pratt (18). It seems evident that the total quantity of blood in the normal body bears a definite relation to the oxygen requirement of the body.

The greater percentage decrease in volume in females than in males is consistent with known

changes in bodily condition taking place with advancing years, particularly in view of the tendency towards obesity occurring in women after the menopause.

The selection of a basis for the determination of normal values for comparative purposes in clinical investigation presents some difficulties. It is apparent that the blood volume of a given

for calculation of normal volume on the basis of height or surface area is shown in Figure 8.

#### CONCLUSIONS

(1) Plasma and total blood volume, venous pressure and blood velocity rates were determined in 49 normal males and 41 normal females.

(2) No relationship exists in normal persons

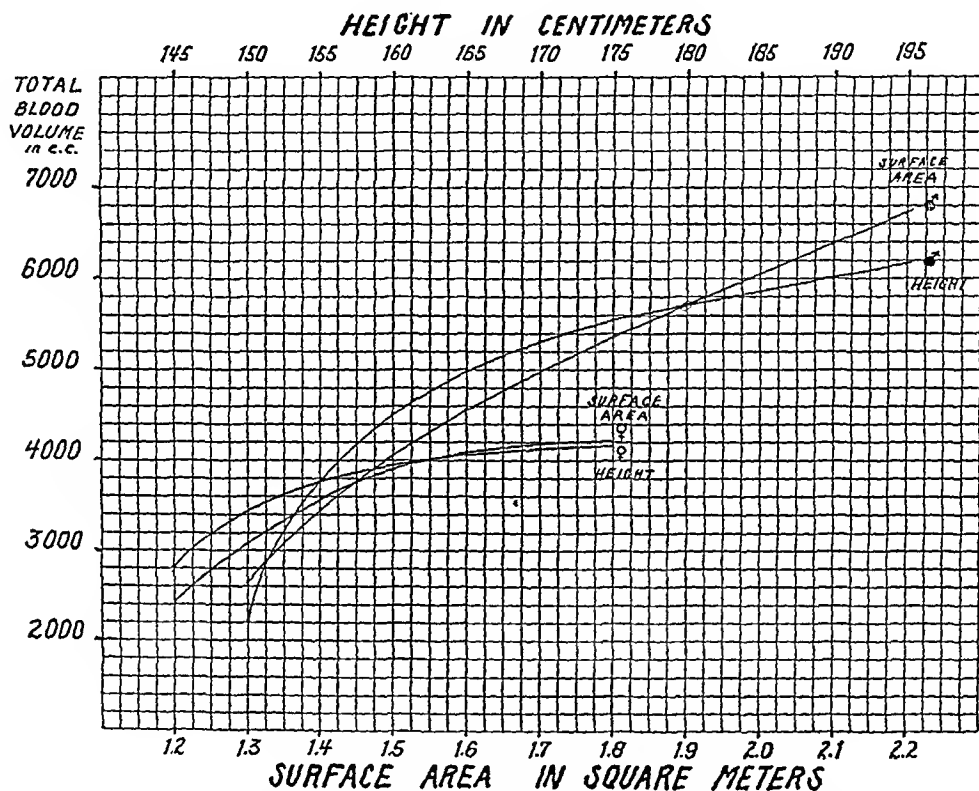


FIG. 8. CHART FOR DETERMINING NORMAL BLOOD VOLUME FOR MEN AND WOMEN ON THE BASIS OF HEIGHT OR SURFACE AREA (AS COMPUTED FROM THE NOMOGRAM OF BOOTHBY AND SANDIFORD)

individual may be predicted on the basis of height, weight or surface area only within wide limits. The average value found for males and females in this series does not reflect differences due to body size. We feel that in a group of patients, the average of normal values based on surface area in those cases exhibiting no marked disturbance in weight to height relationship, and on height in those cases presenting weight changes due to disease, offers a more useful estimate of normal volume than the average values for the entire group of normals herein reported. A chart

between variations in total blood volume, venous pressure and blood velocity rate.

(3) The total blood volume of normal males is greater than that of females, the difference being due to the greater red cell volume of males. The absolute red cell volume of females is less than that of males by a much greater degree than indicated by differences in red cell counts and hematocrit values.

(4) With increasing age there is a decline in the blood volume comparable to decreases in basal metabolic rates and vital capacities.

(5) In comparison to average values, the absolute total blood volume is high in muscular and obese persons, and low in thin individuals; the volume per unit of body weight is high in muscular and in thin individuals and low in obese persons.

(6) The blood volume of normal individuals varies within wide limits. The relationship to height or surface area offers a useful basis for estimation of normal volume in clinical investigation.

We wish to acknowledge our grateful appreciation to Professor Henry A. Christian for advice and encouragement tendered us in this work; to the many members of the staff of the Peter Bent Brigham Hospital for their coöperation in volunteering as subjects, and to Miss Evelyn Berstein for valuable technical assistance.

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# RELATION OF THYROID AND PARATHYROID GLANDS TO CALCIUM AND PHOSPHORUS METABOLISM. STUDY OF A CASE WITH COEXISTENT HYPOPARATHYROIDISM AND HYPERTHYROIDISM

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In a series of investigations on the relation of the thyroid to calcium and phosphorus metabolism Aub and his coworkers (1) have demonstrated that increased thyroid activity is associated with an increased excretion of calcium and phosphorus. In exophthalmic goiter there is an increased output of calcium not only in the urine but in the feces. In normal individuals who are fed thyroid there is a similar increase in calcium and phosphorus excretion, and in myxedema there is a diminished excretion which rises toward normal following thyroid therapy. This increase in negative calcium and phosphorus balance is dissimilar to that produced by overactivity of the parathyroid glands. In hyperparathyroidism the fecal calcium excretion is not increased in the absence of kidney damage (2). The blood levels of both calcium and phosphorus are altered in hyperparathyroidism while in hyperthyroidism they are usually within normal limits. These workers have also demonstrated improvement in tetany of hypoparathyroid origin from thyroid medication with an elevation of the lowered blood calcium level toward that of normal (3). Careful total acid-base balance experiments have excluded in their opinion acidosis as a cause of this altered metabolism (4). Vitamin D deficiency has also been studied as a possible cause and has been considered excluded (5). The conclusion of these workers is that if the blood calcium level is below normal before medication an increased amount of the thyroid hormone releases calcium and phosphorus from the bones with a consequent rise of the blood calcium level and an increased excretion of both calcium and phosphorus by the kidney and large bowel. The usual absence of altered blood levels of both calcium and phosphorus, together with the increased fecal calcium excretion, lead them to believe that in hyper-

thyroidism a concomitant overactivity of the parathyroid glands is not present. These conclusions have recently been challenged by Hansman and Wilson (6) in a series of studies on patients with hyperthyroidism, two of whom also had postoperative hypoparathyroidism. These Australian workers believe that in thyrotoxicosis there is an associated overactivity of the parathyroid glands and that the alterations in calcium and phosphorus metabolism are directly due to the excessive parathyroid secretion.

The appearance of a patient in the Thyroid Clinic with postoperative parathyroid tetany and a recrudescence of her exophthalmic goiter presented us with an unusual opportunity to study the interrelation of the thyroid and parathyroid glands in their control of calcium and phosphorus metabolism.

## METHODS

The metabolic studies of calcium and phosphorus were carried out in periods of three days each, according to the routine developed on the metabolism ward of this hospital by Bauer and Aub (7). Both the dietary and fluid intake were identical for all seven periods except that extra sugar was allowed the patient in Periods V to VII. The diet was neutral in its acid-base contents. The constituents were not actually analyzed but were calculated from previous analyses of standard foodstuffs used on the metabolism ward. The calcium and phosphorus in both blood serum and the excreta were determined by the Fiske methods (8, 9). In order to avoid an increase in inorganic phosphate from organic phosphate (10), the blood for all phosphorus determinations was taken under oil and the determinations started immediately. Magnesium was determined by a modification of the Handy method (11). Phosphatase is recorded in Bodansky units (12). The remaining determinations were made by standard laboratory procedures. The blood chloride and carbon dioxide determinations were made on arterial blood taken under oil.

## SUMMARY OF HISTORY

The observations were carried out on E. M. G., an unmarried woman of twenty-seven. (A complete protocol

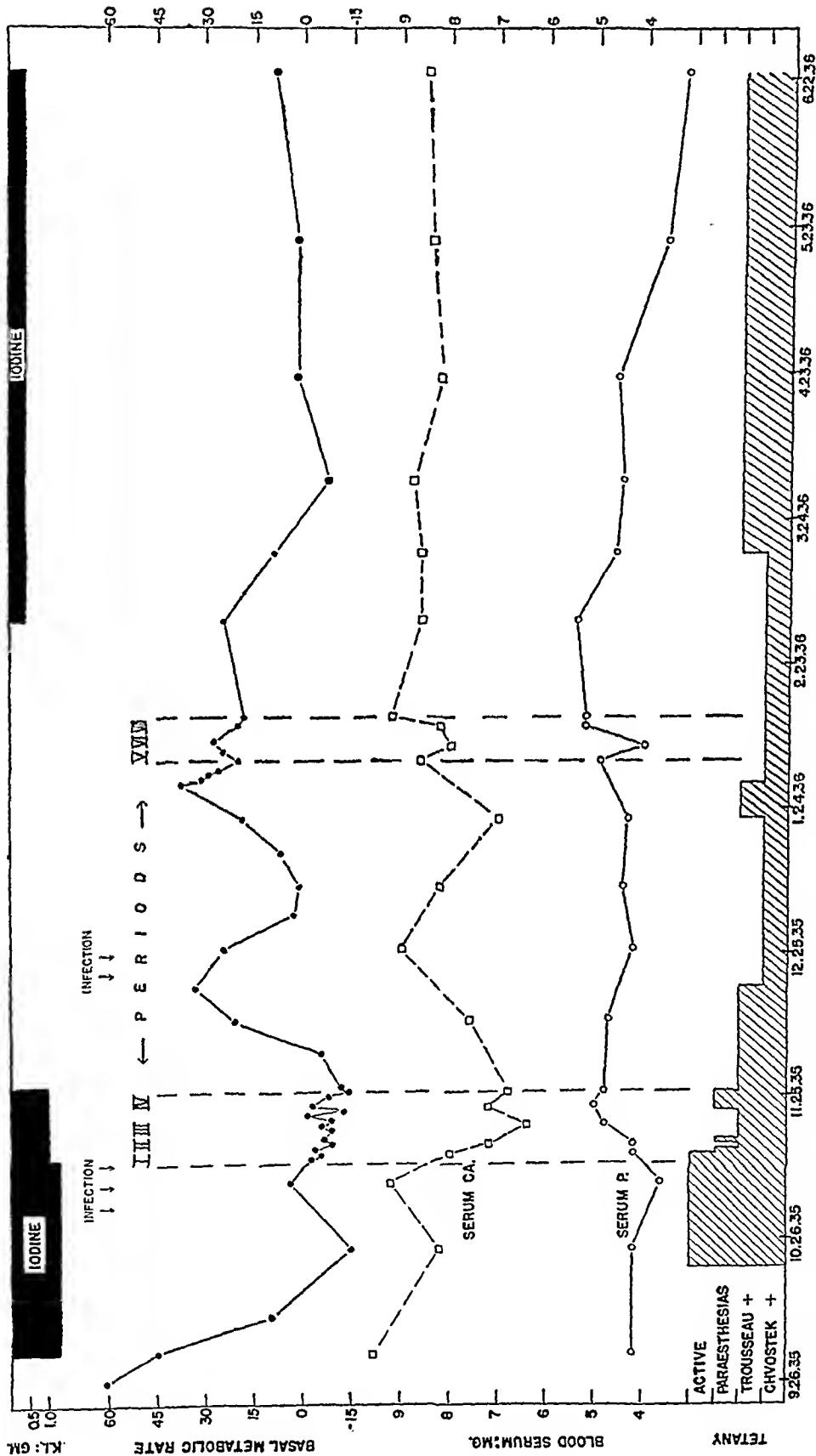


FIG. 1. BASAL METABOLIC RATE, BLOOD SERUM CALCIUM AND PHOSPHORUS, DEGREE OF TETANY AND IODINE ADMINISTRATION FOR ENTIRE NINE MONTHS OF OBSERVATION BY AUTHORS.

The time of the metabolic periods is shown by vertical lines.

Note: Three degrees of latent tetany are distinguishable by physical signs and symptoms. When tetany occurs following operative removal or damage to parathyroid tissue, the earliest clinical indication is a positive Chvostek sign. Occasionally, in normal individuals, the Chvostek sign is positive but where not present normally, it represents the mildest form of latent tetany. If the drop in blood serum calcium continues, the Trousseau sign is next to appear and represents a moderate degree of latent tetany. Paresthesias are usually the third in the progression and indicate impending active tetany.

of the history and findings on this patient is appended and reference should be made to it for details.) She first came to the Clinic in September 1932 with typical hyperthyroidism of moderate severity. Immediately following subtotal thyroidectomy, tetany of parathyroid origin appeared. During the next two and one-half years mild tetany continued to be almost completely controlled by calcium lactate ingestion. A recrudescence of thyrotoxicosis was established in April 1933, and from that time until December 1934 the thyroid activity was held within normal limits by iodine. In order to help control the mild chronic tetany, iodine was discontinued in December 1934 and her metabolic rate slowly rose to +35 over a period of four months. At the end of this rise, it was noted that her tetany had disappeared and calcium medication was omitted. Regrowth of thyroid tissue and increase of thyrotoxicosis continued to occur until on September 26, 1935, her metabolic rate had reached +61 and a full-blown recrudescence of thyrotoxicosis, including exophthalmos, was apparent. At this time she first came under the observation of the authors. It was realized that control of the thyrotoxicosis with iodine was likely to cause an abrupt reappearance of parathyroid tetany. At this point, therefore, calcium and phosphorus studies were started, which will be considered in detail.

Figure 1 shows the changes in metabolic rate during the period of observation by the authors, together with the blood serum calcium and phosphorus and the clinical degree of tetany. At the height of the recrudescence of thyrotoxicosis on October 2, 1935, the serum calcium was 9.6 mgm. and serum phosphorus 4.2 mgm. Clinical signs of tetany were absent. Iodine was started on this date with a fall in metabolic rate to +10 on October 10th, and -15 on October 24th. Completely relieved of her symptoms of thyrotoxicosis and feeling unusually well, on October 20th an attack of tetany suddenly appeared. For the next two weeks, until her entry into the hospital, daily attacks of mild tetany occurred in spite of high calcium medication with viosterol.

On November 6, 1935, the patient entered the metabolism ward for the first series of observations. The thyrotoxicosis was under complete control with iodine by mouth. Potassium iodide, 1 gram, was given daily. Four days before entry, she acquired an acute upper respiratory infection. With the infection she noticed increased tetanic spasms, and, on the day of entry, she was having active tetany.

In spite of the tetany she was placed on the standard low calcium diet for five days preceding the first day of observation. Intravenous and intramuscular calcium gluconate was given in sufficient quantities to control the tetany. Active tetany disappeared coincidentally with the bronchitis and upper respiratory infection, and calcium gluconate was discontinued after the first day of the first period.

During the fifteen days covering the time of the four periods observed during the iodine induced remission, the patient continued to have more or less constant numb-

ness and tingling of the extremities. There were occasional cramps involving the small muscles of the thumbs and also the feet. The Chvostek and Trousseau signs were positive throughout.

Following these first four periods iodine was discontinued, and the patient was allowed to go home on a low calcium diet. The metabolic rate was observed weekly. The thyrotoxicosis recurred promptly. When the metabolic rate was above 30 the patient developed another acute respiratory infection which apparently initiated a spontaneous remission. In two weeks the metabolic rate dropped nearly to normal. There was no evidence that the patient had received iodine in any form. The patient was very cooperative and intelligent and ate only those things allowed in her diet. A study of the only medication taken on her own initiative showed it to contain no iodine. Following this unusual remission the metabolic rate again rose over a period of three weeks to +37 on January 29, 1936. On this day she was again admitted to the metabolism ward where, after a preliminary four days under standard conditions, three three-day periods were observed.

No iodine was given during this entry. Daily determinations showed a metabolic rate continuously 20 per cent above normal. Signs and symptoms of thyrotoxicosis were present. Although she had occasional sensations of numbness, there was no active tetany similar to that observed during the period of observation under iodine control. The Trousseau sign throughout was negative, but of note was a continuously positive Chvostek. The electrical reactions still showed the diminished threshold consistent with tetany.

After the metabolic studies were complete, iodine was again given. A prompt fall in metabolic rate to just below normal, typical of an iodine remission, proved the existence of true thyrotoxicosis. Mild tetany reappeared. Subsequently, the tetany has been controlled with large doses of viosterol and a high calcium, low phosphorus diet. The metabolic rate has remained within normal limits, the patient receiving iodine daily.

#### OBSERVATIONS

*Calcium and phosphorus metabolism.* Figure 2 shows graphically the metabolic balances of calcium and phosphorus. The metabolic rate and the blood serum levels of calcium and phosphorus for each period are given. Table I gives the actual figures for the intake and excretion of calcium and phosphorus, together with the metabolic rate and the excretion of nitrogen and potassium. In the first four periods positive balances of calcium and phosphorus were found. On this standard diet a slightly negative balance of both calcium and phosphorus is observed in normal individuals (13). The urinary excretion of calcium in the normal controls averages 170



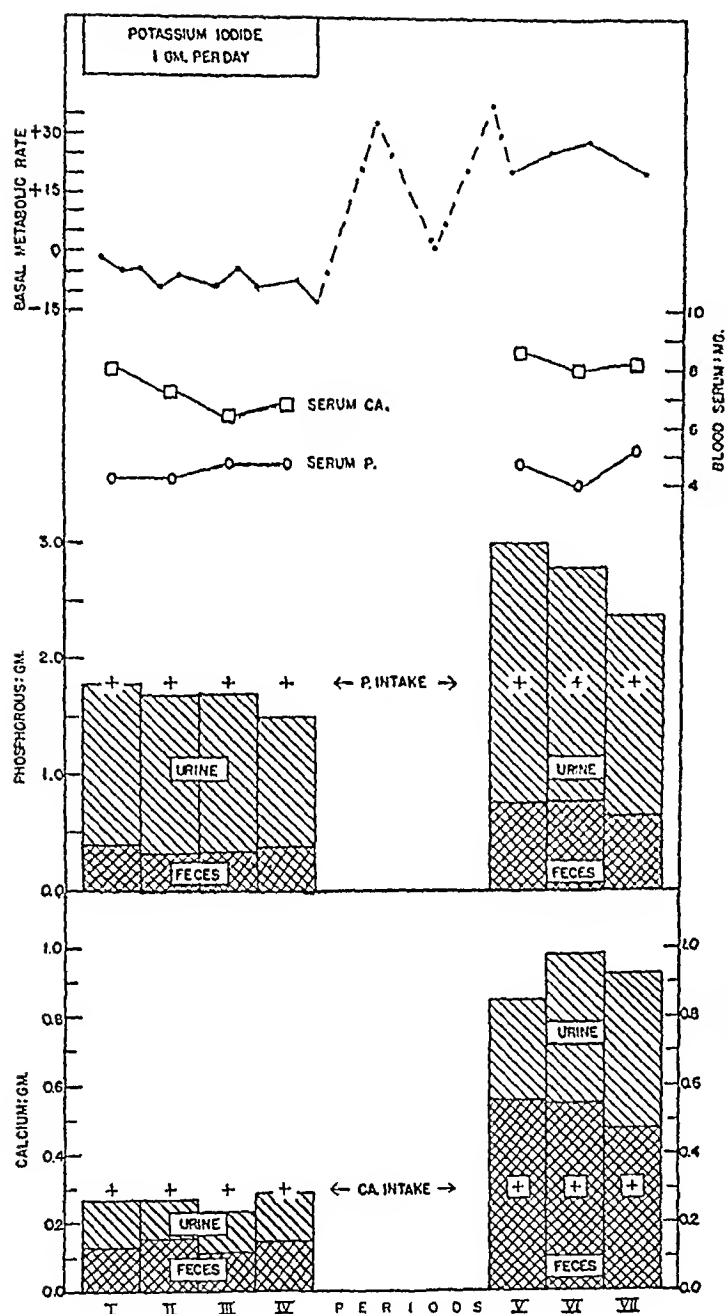


FIG. 2. CALCIUM AND PHOSPHORUS BALANCES, TOGETHER WITH METABOLIC RATE, BLOOD SERUM CALCIUM AND PHOSPHORUS VALUES FOR EACH PERIOD.

mgm. and the fecal excretion 350 mgm. In the controls in nitrogen equilibrium, the negative phosphorus balance is about half that of the calcium. The positive balance of both calcium and phosphorus observed in this patient during the iodine induced remission is characteristic of decreased parathyroid function. This was to be expected since the thyrotoxicosis was completely controlled and active tetany was present. It is interesting that the actual excretions in Period I were as low as in the succeeding three periods in spite of the parenterally administered calcium gluconate used to control the tetany.

The last three Periods, V, VI and VII, observed during the recrudescence of thyrotoxicosis, show abnormally high negative calcium and phosphorus balances. With the same intake of calcium, the total excretion was nearly double that seen in normal controls and represents an increase of 247 per cent over the previous periods. This increase in calcium excretion took place both in the urine and feces. Although the percentage increase in both was about the same, the actual increase was much greater in the feces. The fecal excretion was also nearly double the dietary intake of calcium and it is obvious, therefore, that a true intestinal excretion occurred.

The increased excretion of phosphorus in the last three periods was more than would be expected from the increase in calcium excretion alone. A negative balance of calcium indicates withdrawal of calcium from the body stores. Since the only available store is in the bones (14), where it is deposited as phosphate and carbonate, release of calcium should be accompanied by a proportionate release of phosphate. If apatite (15) is taken as the bone salt, the calcium to phosphorus ratio is 2.26, and the increased calcium excretion would, therefore, account for 290 mgm. of the 1057 mgm. average increase observed in phosphorus excretion per period.

The remainder of the increase in phosphorus excretion found during thyrotoxicosis is quantitatively accounted for by the increased nitrogen excretion of 12.0 grams per period. The dietary intake was identical for all seven periods, except that during the three of increased metabolic rate, sugar and sugar candy in excess of the weighed diet were allowed the patient in the hope of meeting the increased caloric requirement. Although the weight of the patient was essentially maintained during the last three periods, the above increase in urinary nitrogen occurred. That this nitrogen increase was at the expense of body cellular protein is confirmed by the increase in potassium excretion. As with the withdrawal of calcium from bone, so the breakdown of body protein is accompanied by release of phosphorus as well as nitrogen. From the ratio  $N/P = 17.4$ , derived from Benedict's fasting man (16), the nitrogen equivalent of the increased phosphorus excretion equals 689 mgm. This nitrogen equivalent

TABLE I

*Metabolic data (Values in grams per three day period)*

Period	Medication	Phosphorus				Calcium				Nitro- gen	Potassium		Average B.M.R.	Aver- age weight
		Intake	Output		Balance	Intake	Output		Balance	Urinary excre- tion	Output			
			Urine	Feces			Urine	Feces			Urine	Feces		
I	3 grams K.I.	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	per cent	kgm.
II	3 grams K.I.	1.788	1.396	.385	+ .007	.300*	.121	.146	+ .033	20.2	5.114	.702	- 4	57.4
III	3 grams K.I.	1.788	1.366	.323	+ .099	.300	.109	.164	+ .027	19.6	5.114	.508	- 8	57.9
IV	3 grams K.I.	1.788	1.365	.334	+ .089	.300	.118	.118	+ .064	20.2	5.083	.626	- 8	58.3
	3 grams K.I.	1.788	1.118	.385	+ .285	.300	.147	.136	+ .017	17.5	4.500	1.095	-11	57.8
Average	3 grams K.I.	1.788	1.311	.357	+ .120	.300	.124	.141	+ .035	19.4	4.953	.733	- 8	57.9
V	0	1.788	2.280	.740	- 1.232	.300	.304	.555	- .559	32.7	7.036	1.330	+23	54.7
VI	0	1.788	2.030	.751	- .993	.300	.430	.550	- .680	32.3	5.895	1.130	+28	53.9
VII	0	1.788	1.737	.636	- .585	.300	.460	.465	- .625	29.1	5.395	1.210	+20	54.0
Average	0	1.788	2.016	.709	- .937	.300	.398	.523	- .621	31.4	6.109	1.223	+24	54.2

\* This figure represents only the total dietary intake. given intramuscularly which was not included in this total. greater than recorded.

On the first day of this period 1 gram of calcium gluconate was The actual positive balance for the first period was therefore

lent together with the calcium equivalent leaves but 78 mgm. of the increase in phosphorus excretion unaccounted for. This discrepancy is well within the limit of error in a balance experiment.

It is interesting that in this patient the increased phosphorus excretion during thyrotoxicosis occurred in the feces as well as in the urine. In all the patients studied by Aub et al. with either primary thyrotoxicosis or that induced by thyroid extract, the increased excretion of phosphorus was limited to the urine.

*Levels of calcium and phosphorus in the blood serum.* In Figure 1 it will be observed that the blood serum calcium level was in general higher when hyperthyroidism existed than when a normal metabolism had been induced by iodine. Although the curves of the blood calcium level and the metabolic rate are parallel throughout, the blood calcium rose and fell nearly two weeks after the corresponding changes in metabolic rate. A similar delay in change of the serum calcium level behind the metabolic rate was noted by Aub, Albright, Bauer, and Rossmesl (3) in the second of the two patients with hypoparathyroidism treated with thyroxin. At the end of October the delayed fall in blood calcium was in all probability influenced by the high calcium medications given during active tetany and perhaps also by the respiratory infection. Until the end of Janu-

ary 1936, the phosphorus changed inversely to the serum calcium and metabolic rate. During the period of thyrotoxicosis, however, the serum phosphorus rose slightly, parallel to the blood calcium. It is doubtful that the excretion of calcium is dependent upon the total serum calcium level since in Periods I to IV the excretion of calcium is essentially the same while the blood levels varied from 8.0 to 6.4 mgm. The increased excretion in Periods V to VII occurred with a serum calcium level from 8.0 to 8.6 mgm. The average blood phosphorus level for the first four periods was 4.5 mgm. and for the last three, 4.7 mgm. It is equally doubtful that the change in phosphorus excretion could be dependent upon this minor change in the serum phosphate level.

*Tetany.* At the base of Figure 1 the amount of clinical tetany is shown graphically.

Tetany of parathyroid origin may be divided into active and latent. In this patient the tetany was not severe and active tetany on the chart represents merely moderate though painful spasms. Latent tetany is subdivided into three categories according to severity. These are based on observation of the 35 cases of hyperparathyroidism operated upon at this hospital as well as cases of tetany observed following thyroid operation.

In the week immediately preceding Period I,

when active tetany occurred, the blood calcium was above 8.0 mgm. During the first three periods, as the tetany improved, the blood calcium level fell, and when the lowest blood calcium level was reached at 6.4 mgm. in Period III, active tetany and paresthesias had disappeared, only positive Trousseau and Chvostek signs being present. This improvement in tetany with a concurrent fall in blood serum calcium is the reverse of what usually happens in tetany of parathyroprivic origin.

It is currently believed that the tetany associated with diminished parathyroid function is due to a lowered level of the dissociated or ionic calcium of the blood serum and not to any alteration in amount of the protein bound fraction. Direct serum calcium partitions were not done on this patient. In normal individuals indirect evidence of the calcium partition can be obtained from the concentration of serum protein to which the undissociated calcium is bound quantitatively and from the level of the spinal fluid calcium which is believed to represent the filtrable or ionized equivalent of the blood serum. Sufficient determinations of serum protein and spinal fluid calcium were not made to explain the paradoxical relation of total serum calcium to degree of tetany. It will be observed from the figures in Table II that during the period of iodine induced remission, when the tetany was more active, the serum protein was slightly more elevated and the spinal fluid calcium was 1.4 mgm. lower than during thyrotoxicosis.

It is probable that the tetany was influenced by the upper respiratory infection since the active tetany disappeared at the same time as the signs of infection. A similar increase in the severity of tetany of parathyroid origin, induced by an acute upper respiratory infection, has been noticed in the series of cases of hyperparathyroidism from this hospital (17). In one of the two cases of hypoparathyroidism observed by Aub et al. (3), acidosis produced by ammonium chloride improved the tetany. An acidosis however was not found in our patient during the active tetany. The arterial blood carbon dioxide combining power and content, serum chloride and sodium, taken during active tetany, showed no significant changes. In order to exclude tetany due to over-

ventilation, rebreathing was tried during active spasms. No noticeable effect was obtained.

Throughout this study tetany became less intense with an increase of thyroid activity. This improvement was consistent with the slight elevation of the blood calcium during the thyrotoxic state. It is important to note that except at the very beginning of our observations some degree of tetany was always present. From the first appearance of tetany following iodine administration, the Chvostek sign was continuously present, even during the recrudescence of thyrotoxicosis. The patient had no tetany when the metabolic rate was  $+45$  to  $+61$  and the general clinical picture of thyrotoxicosis was moderately severe. When the metabolic observations were made during the reappearance of thyrotoxicosis, not only was the metabolic rate lower than when tetany was absent, but the clinical signs and symptoms of the thyroid disease were milder. During Periods V to VII, had the degree of thyrotoxicosis been more severe, it is probable that tetany might have been completely absent and the blood calcium been further elevated. It is fortunate that the clinical signs of tetany were not completely eliminated by a more active hyperthyroidism since the presence of tetany gives a clue to the degree of function of the parathyroid glands and excludes the parathyroids as the source of the metabolic changes found.

The probability that tetany of hypoparathyroid origin was latent throughout both groups of observations depends not merely upon the clinical signs but also on the electrical threshold reactions. On November 12, 1935, Period I, 1 milliamperes was required on closing for face and eyelid muscles and 2 milliamperes for the extensor digitorum communis. Opening currents were 3 and 4 milliamperes respectively. Two normal controls checked at the same time required an average of double this amount. On March 4, 1936, when the metabolic rate was  $+25$ , the responses of the face and forearm muscles were obtained with currents actually somewhat lower than at the previous observations.

#### DISCUSSION

The studies of the calcium and phosphorus balance made on this patient confirm the findings of

TABLE II  
Blood and spinal fluid data during metabolic studies in Patient E. M. G.

Date	Pe- riod	Medication	B.M.R.	Weight	Tetany	Blood serum							Whole blood				Spinal fluid			
						Ca	Phos- phorus	Phospha- tase	Pro- tein	Mg	Cl	Na	CO <sub>2</sub> com- bin- ing power	CO <sub>2</sub> con- tent	Sugar	Non- protein nitro- gen	Ca	Phos- phorus	Cl	Mg
						mgm.	mgm.	units	per cent	mgm.	m. eq.	m. eq.	volumes per cent	volumes per cent	mgm.	mgm.	m. eq.	mgm.		
November 7, 1935		1 gram K.I. Calcium lactate	+ 4	56.9	+++	9.2	3.6	6.91			107	55.6								
November 13, 1935	I	1 gram K.I.	— 5	58.0	+++	8.0	4.2	5.41												
November 15, 1935	I	1 gram K.I.	— 9	58.0	+++	7.2	4.2													
November 16, 1935	II	1 gram K.I.	— 7	57.7	+++				6.9	3.0	103	53.4	42.9	103			1.5	1.8		
November 19, 1935	III	1 gram K.I.	— 6	58.9	+++	6.4	4.8	6.90												
November 20, 1935	III	1 gram K.I.	— 9	58.0	+++															
November 23, 1935	IV	1 gram K.I.	— 3	57.8	+++	7.2	5.0	6.70	5.9							16				
November 26, 1935	IV	1 gram K.I.	— 14	57.8	+++	6.8	4.8	6.10	6.5							22				
February 3, 1936	V	1 gram K.I.	+20	54.8	+++	8.6	4.9	4.80	6.1							18				
February 6, 1936	VI		+20		+++	8.0	4.0			106		56.6	47.4				1.5			
February 10, 1936	VII		+20	54.0	+++	8.2	5.2	5.10			134					28	4.6			

Aub and his coworkers. The marked increase in calcium and phosphorus excretion to above normal, occurring during the time of increased metabolic rate with signs of thyrotoxicosis and continued tetany, lends substantial support to the belief that the increased excretion in hyperthyroidism is not due to a concomitant overactivity of the parathyroid glands. It is probable that the parathyroid function was still somewhat below normal although the tetany was improved and the serum calcium level slightly raised toward normal by the increased thyroid function. A positive Chvostek sign and diminished electrical threshold response are not proof of hypoparathyroidism. It is, however, unlikely since the blood serum calcium was still below normal that any overactivity of the parathyroids occurred. This rise in blood calcium and improvement in tetany was observed by Aub et al. (3) upon giving thyroid extract to patients with hypoparathyroidism.

Thyroid extract does not duplicate the disease of hyperthyroidism. Whatever the stimulus is which produces thyroid hyperplasia and increased excretion of the thyroid hormone, be it the anterior pituitary or excessive cervical sympathetic stimulation directly to the thyroid, this stimulus probably is not set in motion when thyroid extract or thyroxin is administered. It is possible that the same stimulus which causes overactivity of the thyroid gland in hyperthyroidism might cause overactivity of the parathyroids. Administration of thyroid extract or thyroxin is essentially a replacement therapy of the thyroid alone and would not necessarily stimulate parathyroid secretion. In the patient reported in this study the increased metabolism occurred from endogenous thyroid hormone. The production of the increased thyroid activity was caused by the stimulus of the disease. If the parathyroid glands are stimulated to overactivity by the disease stimulus in hyperthyroidism, increased activity of the parathyroid glands should have occurred in this patient. It is possible that slight increase in activity of the remaining parathyroid tissue did occur, but if it did, the total parathyroid activity was in all probability still below normal since tetany and a subnormal blood calcium persisted. Any change in the calcium and phosphorus me-

tabolism due to change in the parathyroid activity would still have been, therefore, in the range of hypoparathyroidism. The shift of the excretions to amounts well above normal which did occur with the recrudescence of the hyperthyroidism, the blood calcium remaining below normal, is certainly not characteristic of hyperparathyroidism, and the contentions of Hansman and Wilson (6) are not upheld.

It is still not proven that the entire inorganic metabolic change is due to the altered thyroid activity since the stimulus of the disease in itself might be responsible for part. If the pituitary were primarily responsible for hyperthyroidism, as is currently held by some workers, the increased negative balances of calcium and phosphorus might be due directly to increased anterior pituitary secretion.

Aub (18) has recently shown that pituitary basophilism with osteoporosis is associated with an increased output of calcium and phosphorus in the urine. This abnormal excretion returns to normal following x-ray treatment of the pituitary with relief of the disease. He found no increase in the fecal excretion. Aub (19) has studied cases of acromegaly in which he found an increased negative calcium and phosphorus balance, the abnormal excretion being almost entirely limited to the urine. Calcium and phosphorus metabolism of a patient with adrenal cortical carcinoma simulating pituitary basophilism has been studied in this hospital by Albright (20). As in the pituitary diseases, an increased excretion of calcium and phosphorus in the urine but not in the feces was found. In cases both of pituitary basophilism and of adrenal cortical carcinoma the metabolic rate was below normal. The cause of the abnormal excretion was, therefore, probably not increased thyroid activity. In acromegaly there is at times increased thyroid activity, and this might in part explain the abnormal excretion in that disease. The occurrence of abnormal calcium and phosphorus metabolism associated not only with overactivity of the parathyroid glands but also of the thyroid, anterior pituitary and adrenal cortex, suggests that calcium metabolism like carbohydrate metabolism is influenced by a balance between various endocrine glands.

As the knowledge of the parathyroid glands

developed it became almost an assumption that their function was the control of calcium within the body. The first challenge of this assumption was the demonstration by Aub and his coworkers of the profound effect upon calcium and phosphorus produced by changes in thyroid function. The specific rôles of the various hormones affecting calcium or phosphorus are doubtless different even though the effect upon the total metabolic change may be the same. To date, only two outstanding differences in the function of these glands have been demonstrated. The first is the control of the blood levels of calcium and phosphorus by parathyroid activity. The second is the increased fecal excretion in hyperthyroidism. Only in hyperparathyroidism with marked renal damage is a similar increase in the excretion of calcium found in the feces. In this condition, the kidney apparently is no longer able to take care of the abnormal outflow of calcium, and the assumption by the bowel of this task represents an abnormality of the disease itself. The demonstration of an abnormal balance of calcium or phosphorus no longer points, therefore, to one specific disease entity.

In the patient reported in this paper an increased phosphorus excretion as well as that of calcium, demonstrated during active hyperthyroidism, has been found in the feces for the first time. Its meaning or significance is not evident.

#### SUMMARY

A study of the calcium and phosphorus metabolism has been made in a patient suffering from recurrent thyrotoxicosis and postoperative parathyroid tetany. When the metabolic rate was maintained within normal limits with iodine medication the calcium and phosphorus metabolic balances were characteristic of parathyroid tetany. During hyperthyroidism a marked increase in the negative balance of calcium and phosphorus beyond normal limits was found even though signs of diminished parathyroid activity continued.

Increased thyroid activity was followed by a rise of the subnormal blood serum calcium level toward that of normal and a decrease in the signs of tetany. The reverse occurred with a decrease in thyroid function.

An acute upper respiratory infection was as-

sociated with an increase in the amount of clinical tetany and also with a spontaneous remission of the thyrotoxicosis.

Because of the probably continued subnormal activity of the parathyroid glands it is concluded that the changes of calcium and phosphorus metabolism were due to the changes in thyroid function. Further support is given to the belief that the changes in calcium metabolism previously reported in hyperthyroidism are not due to a concomitant overactivity of the parathyroid glands.

A discussion of the relation of the parathyroid, thyroid, anterior pituitary and adrenal cortical glands in calcium metabolism is given.

#### PROTOCOL

E. M. G., female, age 23, M. G. H. number 323612, metabolism number 10759, entered the hospital August 31, 1932, complaining of painless swelling of the neck of one month's duration. During the past year she had noted slight, gradually increasing nervousness. Huskiness and occasional cough had been noted for two months. One month before entry patient had had onset of slight dyspnea on exertion but no palpitation. She had been told her eyes were stary. She had always perspired a good deal; warm weather was well tolerated. She had noted no tremor.

Four years ago following tonsillectomy patient noticed a swelling of her neck which disappeared without treatment. Two years ago and again one year ago antrum had been operated upon. Since second antrum operation she had gained 18 pounds in weight. There have been occasional morning headaches and regular nocturia of one time. Catamenia was regular. Poor financial situation, otherwise past and family histories were non-contributory.

Examination showed an alert young woman with warm moist skin; no eye prominence or lid lag but definite stare. Thyroid was large, uniform, soft, with both thrill and bruit. There was slight tremor of extended fingers, heart was normal and blood pressure 150/80.

Examinations of blood, urine and stool were essentially negative.

X-ray of chest showed shadow of thyroid extending downward beneath manubrium; trachea not displaced or narrowed. Lung markings were prominent with no evidence of consolidation; heart shadow was not remarkable.

Basal metabolic rate on entry was +50 per cent, dropping on rest alone to +39 and +35. Weight was 55.7 kgm. After iodine the rate dropped to +3 by September 10th.

September 12, 1932, subtotal thyroidectomy was performed. A radical excision is recorded. Pathological report: marked hyperplasia of the epithelial elements;

unusually little lymphoid infiltration and no definite evidence of involution. Tissue weighed 95 grams.

Two days after operation carpopedal spasm and paresthesias appeared. Chvostek and Trousseau signs were positive. The following morning serum calcium was 6.3 mgm., serum phosphorus 4.2 mgm. A high calcium intake was started. Signs of active tetany were absent two days later. Trousseau and Chvostek signs remained positive. On ninth postoperative day metabolic rate was  $-16$ . Patient was discharged from hospital September 22, 1932; diagnosis, exophthalmic goiter; complication, tetany.

#### *Follow-up observations*

In the interval from October 26, 1932, to September 26, 1935, the patient made the following sixteen visits to the Outpatient Thyroid Clinic:

October 26, 1932, B. M. R. was  $+5$ , Chvostek plus, Trousseau plus, serum calcium 7.7 mgm., and serum phosphorus 4.2 mgm. Patient was placed on calcium lactate by mouth.

December 8, 1932, B. M. R. was  $-7$ . There were occasional paresthesias and cramps in the right arm; Chvostek and Trousseau were negative.

February 9, 1933, B. M. R. was  $+8$ . Patient was still having cramps and paresthesias in spite of calcium lactate. Pyramidal lobe and small irregular lump in region of left thyroid lobe were palpable. Serum calcium was 8.3 mgm., and serum phosphorus 3.5 mgm.

April 20, 1933, B. M. R. was  $+17$ . Patient was still taking calcium lactate. There were no symptoms of tetany, Chvostek was positive. Lugol's, minims 3 daily, was started.

June 22, 1933, B. M. R. was  $-14$ ; weight 58.7 kgm.

September 14, 1933, B. M. R. was  $+1$ . Patient was still taking calcium lactate and Lugol's.

December 14, 1933, B. M. R. was  $+10$ , serum calcium 8.7 mgm., and serum phosphorus 3.4 mgm.

March 15, 1934, B. M. R. was  $+20$ .

June 14, 1934, B. M. R. was  $+9$ . Patient complains of recurrent nervousness, occasional numbness of left hand. Chvostek was negative and Trousseau strongly positive in two minutes. Iodine was increased, calcium lactate continued.

September 13, 1934, B. M. R. was  $-12$ , weight 53.5 kgm. Patient was less nervous with no symptoms of tetany. Chvostek and Trousseau were negative. Thyroid nodule on left was recorded as  $3 \times 3$  cm., on right  $1 \times 1$  cm. with 0.5 cm. pyramidal lobe. There was slight exophthalmos and no lid lag.

December 13, 1934, B. M. R. was  $+6$ . Only 1 to 2 drops of potassium iodide a week had been taken since last visit; calcium was continued.

February 14, 1935, B. M. R. was  $+26$ , weight 56.0 kgm. No iodine had been taken since last visit; patient has continued taking calcium lactate. Chvostek and Trousseau were negative. Impression, tetany free, recurrent toxicity; omit calcium.

March 14, 1935, B. M. R. was  $+36$ .

April 11, 1935, B. M. R. was  $+22$ . Tetany free.

May 23, 1935, B. M. R. was  $+39$ . Patient was increasingly nervous, Chvostek and Trousseau were negative.

July 25, 1935, B. M. R. was  $+26$ , weight 51.6 kgm.

September 26, 1935, B. M. R. was  $+61$ , pulse 126, weight 56.1 kgm. At this visit the patient was seen for the first time by the authors. She was definitely jumpy, overactive, and presented the picture of full-blown thyrotoxicosis. There was definite tremor. In contrast to the time of operation there was now a moderate but definite exophthalmos with no lid lag, a normal convergence, but a definite stare. A regrowth of thyroid tissue on both sides, more marked at the left upper pole, was evident. Chvostek and Trousseau signs were negative.

October 2, 1935, B. M. R. was  $+45$ , serum calcium 9.6 mgm., and serum phosphorus 4.2 mgm. Patient was started on iodine.

October 10, 1935, B. M. R. was  $+10$ , pulse 96.

October 24, 1935, B. M. R. was  $-15$ , pulse 60. One week ago patient was feeling wonderfully. She had not realized how nervous she had been; felt much quieter now. Four days ago she had had a sudden attack of tetany preceded by headache and has had an attack of carpopedal spasm every day since. Examination showed a definite change in the general picture since iodine medication. Patient was quiet and reposed, tremor was much diminished, Chvostek moderately positive, Trousseau positive at  $1' 40''$ , serum calcium 8.2 mgm. and serum phosphorus 4.2 mgm.

#### *Second admission*

Patient was admitted to the metabolism ward for study on November 6, 1935. Four days before entry she had contracted a cold which "settled" in her chest. There has been a productive cough with chest pain. In addition to points already covered in notes from the outpatient clinic, her history contains the following items.

She has been subject to head colds, last and most severe of which occurred in May 1935. At this time she had a temperature of  $103^{\circ}$  F. and was in bed for six days. Following this she was quite run down. She has had increasing headaches of late; generalized, never severe, averaging about two a week for the last 2 months, and having also preceded the recent attacks of tetany. During the past 5 months, there have been occasional attacks of blurred vision lasting about ten minutes; no diplopia. Shortness of breath, complained of in first admission, has been noticed again recently; no palpitation. On two occasions recently she has been awakened at night by a sense of suffocation which has passed off rapidly. There was no edema and only occasional nocturia. Catamenia was regular with no change in flow or menses. Memory was good. Lately patient has had to restrain herself from becoming irritated with her family.

*Dietary history.* Patient has always liked milk and for past year has averaged  $1\frac{1}{2}$  quarts a day, a moderate amount of butter, cheese once a week, and occasionally nuts. She eats green vegetables and fruit daily.

*Physical examination.* Patient was a well developed,



well nourished, composed woman with moderate exophthalmos with stare, no lid lag, and slight fine tremor. There was a thyroidectomy scar with thyroid tissue definitely palpable. Right lobe measured  $3 \times 3 \times 2$  cm. At left upper pole there was a rounded mass, 3 cm. in diameter. The body of left lobe was slightly enlarged. No thrill or bruit was present. There were coarse râles at left base posteriorly consistent with bronchitis. On dorsum of hands between first and second metacarpals there were fibrillary twitchings; no muscular twitchings were seen elsewhere. Chvostek 2+, Trousseau positive at 45", associated with considerable pain. No lens opacities were visible. Impression: hypoparathyroidism; iodine remission of thyrotoxicosis; acute upper respiratory infection with bronchitis.

November 7, 1935, patient had an attack of tetany with carpopedal spasm and spasm of small muscles of feet. Calcium gluconate, 1 gram, was given intravenously and 2 grams more intramuscularly. Tetany continued until after the third injection.

November 8, 1935, tetany recurred; patient was slightly apprehensive and uncoöperative, suggesting mental change of tetany. Calcium gluconate, 2 grams, was injected intramuscularly with relief of spasm. Patient's coughing was still present.

On November 9, 10, and 11, daily attacks of tetany were relieved with 1 gram calcium gluconate intramuscularly. Mental outlook was improved.

November 12, 1935, electrical reactions taken by Dr. Robert Schwab showed 50 per cent lowered electrical threshold during latent tetany. Active tetany occurred later with cramps in arms and calf muscles, paresthesias. Chvostek continued strongly positive. Calcium gluconate, 2 grams, was injected intramuscularly with subsequent relief. The first period was started November 12, 1935.

November 13, 1935, upper respiratory infection had subsided and chest was clear. Chvostek and Trousseau signs remained strongly positive but there was no active tetany; patient was more cheerful. No further calcium gluconate was administered.

From November 14 to 27 patient spent most of her time in bed but was allowed up for short periods. She had no further active tetanic spasms but from time to time had paresthesias and some pain in small muscles of feet. Chvostek and Trousseau signs remained positive throughout.

X-rays, November 26, 1935: density of bones of hands and forearms normal, normal trabeculation; skull showed some calcification in dura at the vertex; bones of spine and pelvis showed nothing unusual.

November 27, discharged home. Patient was still having mild paresthesias in both hands and feet but no definite tetanic spasms. No signs of thyrotoxicosis were present except residual exophthalmos and palpable goiter. Iodine was omitted in order to allow thyrotoxicosis to reappear. A low calcium intake was to be continued.

#### *Observations in Outpatient Metabolism Clinic*

December 4, 1935, B. M. R. was —6, pulse 66, weight 57.7 kgm.

December 11, B. M. R. was +21, pulse 96. Patient was still having occasional cramps in feet; both hands felt tight yesterday, no numbness or tingling; occasional palpitation; no nervousness or excitement. Serum calcium was 7.6 mgm., serum phosphorus 4.7 mgm., and serum phosphatase 6.0 units.

December 18, B. M. R. was +33, pulse 114. Cramps in feet have continued. She had been active a whole week and felt fidgety. There was definite increase in perspiration; she was hungry and eating ravenously. Chvostek was present but diminished; Trousseau could not be obtained in two minutes. Her hands were clammy with increased tremor. There was definite lid lag, and forehead did not wrinkle. Thyroid remained the same size; bruit and thrill were present over nodule on left side.

December 26, 1935, B. M. R. was +25, pulse 126, weight 54.1 kgm. Five days ago patient contracted a "cold" with cough and chest pain for last two days. There was no further tetany or tingling; no further blurring of vision; Chvostek faint. Serum calcium was 9.0 mgm., serum phosphorus 4.2 mgm., and phosphatase 5.0 units. She is continuing on a low calcium diet.

January 2, 1935, B. M. R. was +3, pulse 78. Upper respiratory infection has been relieved. There was no nervousness or sweating. Appetite was as good as ever. The only medication has been vaporub; no foods have been eaten which might have contained iodine. Faint Chvostek was present on right side only, no Trousseau after 1½ minutes.

January 8, 1936, B. M. R. was +1, pulse 60; there was definite decrease in appetite. Patient menstruated for first time in 8 weeks. Serum calcium was 8.2 mgm., phosphorus 4.4 mgm., and phosphatase 4.7 units.

January 15, B. M. R. was +7, Chvostek positive, and Trousseau negative.

January 22, B. M. R. was +19, pulse 78, weight 55.3 kgm. Patient was still having cramps in forearms, fingers and feet, was occasionally dizzy, and feels "touchy." Her appetite was excellent. Chvostek was +2. Trousseau positive in 45". Serum calcium was 7.0 mgm., phosphorus 4.3 mgm., and phosphatase 4.9 units.

January 29, B. M. R. was +37.

#### *Third hospital admission*

Patient was admitted to the metabolism ward for study on January 29, 1936. Since discharge from hospital nine weeks ago she had coöperated well in adhering to a low calcium diet with intake of between 200 and 300 mgm. per day. She had taken no iodine since the last visit and has now completely recovered from the upper respiratory infection contracted five weeks ago. At this admission patient complained of some irritability and nervousness which have appeared during the past week. She had not been bothered by cold weather and had slept well. There had been a return of her ravenous appetite; one attack of diarrhea four days ago. Two weeks ago she had had one attack of blurred vision lasting a few minutes.



Examination showed a slightly excited, well nourished patient. Exophthalmos was present in same degree as at the previous admission; stare was visible. At times a lid lag was present and a normal convergence. No lens opacities were seen with either slit lamp or ophthalmoscope. There was a fine tremor, the palms of the hands were moist. The thyroid nodules were approximately the same size as at her previous entry. Thrill and bruit were absent, Chvostek positive, Trousseau negative.

The patient was immediately placed on the same standard neutral low calcium diet with same control fluid intake as at the second admission. Three metabolic periods of three days each were observed after a preliminary five days of the diet. During the fifteen days of ward study she showed signs of continued thyrotoxicosis. Except for last four days there was a fever between 90 and 100 every afternoon. There was no evidence of infection. The pulse rate remained elevated between 100 and 125. Except for two occasions, the Chvostek sign was positive during this admission, varying from faintly to strongly positive. Although the Trousseau sign was negative throughout, there were occasional complaints of tingling and slight cramps in both hands and feet. There was one dizzy spell; no blurred vision or headache. She had a normal menstrual period immediately after entry. Routine blood and urine studies were negative.

Patient was discharged February 12, 1936. Diagnosis: recurrent thyrotoxicosis, hypoparathyroidism with latent and mild tetany.

#### *Follow-up observations*

March 4, 1936, B. M. R. was +25, pulse 102, weight 56.3 kgm. Patient had been active, not minding the unusually cold weather, and had gained weight with a ravenous appetite. Examination on this admission was the same as during her third hospital admission. Chvostek was positive and Trousseau negative after 1½ minutes. Electrical reactions showed that 2 milliamperes less current were required to obtain a response than on November 12, 1935. Serum calcium was 8.6 mgm., phosphorus 5.4 mgm., and phosphatase 3.0 units. Patient was started on Lugol's solution.

March 18, 1936, B. M. R. was +9, pulse 90. There were mild symptoms of tetany; Chvostek and Trousseau signs were positive. Serum calcium was 8.6 mgm. and phosphorus 4.6 mgm. Patient was started on viosterol, 50 drops a day, and was to continue on a low calcium diet and Lugol's solution.

April 1, 1936, B. M. R. was -7, pulse 72. Symptoms of tetany were less than at her last visit. Residual exophthalmos was present but there were no signs of active thyrotoxicosis. Chvostek faintly positive and Trousseau positive at 1' 30", produced less active cramp than on March 18. Serum calcium was 8.8 mgm., and phosphorus 4.5 mgm.

April 22, B. M. R. was +2, pulse 72, weight 58.3 kgm. Serum calcium was 8.2 mgm., phosphorus 4.6 mgm.

May 20, B. M. R. was +2, pulse 78, weight 56.2 kgm. Serum calcium was 8.4 mgm., phosphorus 3.6 mgm.

June 24, B. M. R. was +9, pulse 84, weight 54.1 kgm. Serum calcium was 8.5 mgm., phosphorus 3.2 mgm. There were no symptoms of thyrotoxicosis, but occasional paresthesias. Trousseau was positive in 45". There was no evidence of cataract. Patient has been continuing medication of cod liver oil, calcium lactate and iodine.

September 23, B. M. R. was +7, pulse 84, weight 53.1 kgm.

October 22, 1936, B. M. R. was +5, pulse 78, weight 52.5 kgm. Just before onset of catamenia, two weeks ago, patient had questionable mild active tetany in right hand; Trousseau was negative.

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# GASTRO-INTESTINAL STUDIES. VII. THE EXCRETION OF XYLOSE IN PERNICIOUS ANEMIA

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Since the advent of liver therapy in the treatment of pernicious anemia it has been known that some patients require greater amounts of potent material than others. Beebe and Lewis (1), and Fouts and Zerfas (2) have shown that patients with a higher incidence of complications, arteriosclerosis, and moderate to advanced involvement of the central nervous system, require on the average a greater daily amount of the active principle in the liver to keep the blood and color index near normal than do those not having these complications. The demonstration by Gänsslen (3) and by Castle and Taylor (4), and the subsequent universal verification of their findings, that liver extract administered parenterally was many times more potent than liver extract given by mouth, led to the general acceptance of the theory that failure in absorption of the active principle from the gastro-intestinal tract accounts in the main for increases in the requirement of liver extract. Heath and Fullerton (5) have studied the rate of absorption of iodine and glycine in an attempt to develop a test for absorption. They concluded that the delay in appearance of iodine in saliva after the ingestion of 0.25 gram of potassium iodide is probably a rough measure of the absorptive ability of the upper small intestine. The appearance time was delayed in 7 of 14 patients who had pernicious anemia. They also concluded that the amino-acid nitrogen content of the blood following the ingestion of 25 grams of glycine gave no useful information regarding the rate of absorption from the gastro-intestinal tract. We, likewise, gained no worthwhile information from the determination of blood amino-acid nitrogen following the oral administration of large amounts of liver extract. Following this failure, we studied the excretion of orally administered xylose, since a consideration of its physical and biochemical properties made it appear that xylose would be a useful substance for determining the absorptive ability of the intestinal

tract. This sugar is not metabolized (6). It apparently passes through the liver unchanged (7), and is eliminated from the body by the kidney (7, 8). It is not reabsorbed by the renal tubules (9) and can be recovered with accuracy in the urine and the blood. Because of these properties, the excretion of xylose has likewise been used as an index of kidney function (7, 8).

Although Keith, Power, and Peterson (10) found no difference in xylose clearances in two normal individuals when the xylose was administered by mouth and when administered by vein, we felt that changes in absorption from the gastro-intestinal tract might influence the excretion of orally administered xylose. It seemed reasonable to assume that any marked deviation of the excretion of xylose from that anticipated by the study of the kidney function would be due to changes in the absorption from the gastro-intestinal tract.

Fishberg (11) has shown that xylose injected into the blood stream disappears at a rate proportionate to the actual momentary concentration of nonfermentable reducing substances in the blood. In addition, Fishberg and Friedfeld (7, 12) have shown that following the oral administration of xylose to patients who had normal kidney function, the curve of nonfermentable reducing substances in the blood approaches its normal value after 5 hours, while, in patients with impaired renal function, the curve of nonfermentable reducing substance continues upward, resulting in a definite retention of xylose in the blood at the end of 5 hours. Therefore, in a number of the patients, blood xylose curves were determined in addition to urine xylose. It was assumed that some additional information on absorption might be gained from comparison of the excretion in the urine and the retention in the blood even in cases with poor renal function. Patients having low excretion and low blood xylose curves would

have to be considered to have poor absorption even if the kidney function was low.

One of the main objections to the use of xylose in absorption studies on pernicious anemia patients was that it was probably of a much smaller molecular size than the active principle of the liver. Xylose has a molecular weight of 150, while it has been estimated by Dakin, Ungley, and West (13) that the molecular weight of the active principle in liver is slightly less than 5,000. However, at the present time, we do not know of a material having the desirable properties of xylose which also has a molecular weight similar to that of the active principle. We are therefore reporting our results on our findings as to the absorption of xylose in patients who have pernicious anemia and in three patients having similar blood pictures. Many of the patients studied have been followed by this department for a sufficient length of time for us to know their status as to maintenance dosage of liver extract.

#### METHODS

The pernicious anemia patients tested have been followed by this department for varying periods of time up to nine years. The patients classified as to maintenance dosage of liver extract have been followed for at least 18 months. The majority, however, have been followed for a much longer period. The patients able to maintain normal red blood cell counts while taking 3 vials of liver extract or 12 capsules of "Extralin" (Liver-Stomach Concentrate, Lilly) or less were considered as easy to maintain. Those requiring liver extract by injection were classified as difficult to maintain. The normal individuals tested were young healthy adults. None of the patients had diarrhea at the time of examination.

On the day of the test the subject's breakfast was limited to dry toast and one cup of coffee. Approximately 1 hour after breakfast they ingested 25 grams of xylose dissolved in water to which lemon juice had been added to mask the unpleasant taste of the xylose. Twenty-five grams of xylose were used as it was found that 50 grams often produced a diarrhea which might interfere with absorption. The xylose in the 5 succeeding hourly urine specimens was determined by the method described by West and Peterson (14) in which the non-sugar reducing

material was removed by a  $\text{H}_2\text{SO}_4\text{-BaCO}_3$  precipitation and the reducing sugars determined by the Shaffer-Somogyi (15) reagent after treatment with a yeast suspension. A standard curve prepared with known amounts of xylose was used to convert the titration figures to xylose.

The xylose in the blood was determined by the Shaffer-Somogyi (15) method on filtrates prepared by zinc hydrochloride precipitation (Somogyi (16)). The fermentable sugars were removed by a yeast suspension as before. Van Slyke's (17) method was used for the urea clearance determinations.

#### RESULTS

The 8 normal individuals in 5 hours excreted an average of 4.68 grams (4.26 to 5.33 grams)

TABLE I  
*Laboratory and clinical findings in 48 patients with pernicious anemia and in 3 patients with blood pictures of pernicious anemia with free hydrochloric acid in gastric contents*

Case number	Age	Red blood cells	Hemoglobin	Xylose excreted in 5 hours	Urea clearance	Involvement of central nervous system	Complications	Maintenance
	years	millions	per cent	grams	per cent normal			
1	60	4.7	97	5.29*	69*	—	+	D†
2	67	5.46	101	3.19*	82	+++	—	D
3	45	4.64	107	4.71*	129*	—	—	D
4	40	5.45	94	5.08	129	—	+	D
5	31	4.73	72	5.76	77	—	+	D
6	67	5.01	101	2.94	76	+++	+	D
7	68	4.72	97	3.13	32	+++	++	D
8	52	4.54	97	3.52	36	—	++	D
	52	4.13	92	3.42	44*	—	++	D
9	61	5.57	129	5.34*	84*	—	+	E†
10	69	5.45	105	4.16	55	+++	+	E
11	59	4.91	78	5.80	95	—	—	E
12	64	5.46	191	7.02	—	—	—	D
	64	3.06	74	5.68	77	—	—	D
	65	2.03	51	5.64	115	—	—	D
13	72	5.81	97	2.65*	66*	++	+	E
14	41	5.45	111	6.68*	156*	—	—	E
15	43	5.40	97	6.42	72*	—	—	E
16	59	4.64	107	3.10	85	—	—	E
17	57	4.79	103	2.71*	59*	—	—	E
18	47	4.56	103	5.96	120	—	—	E
19	63	4.74	106	5.11	108*	+++	—	E
20	62	6.00	113	6.23	94	—	+	E
21	36	5.00	86	2.98*	45*	++	+	D
	35	3.26	84	2.93	46	++	+	D
22	56	5.41	113	2.94	70	+++	+	D
	56	2.19	65	2.90	60	+++	+	D
23	67	4.58	94	2.86	52	+++	+	D
	66	2.95	79	2.52	51	+++	+	D
24	57	5.28	120	5.69	105	—	—	E
	56	2.17	50	3.95	79	—	—	E

\* Average of two tests.

† D = difficult to maintain normal red blood cell count—i.e. require liver extract by injection.

‡ E = easy to maintain normal red blood cell count on oral liver.

TABLE I—Continued

Case number	Age	Red blood cells	Hemoglobin	Xylose excreted in 5 hours	Urea clearance	Involvement of central nervous system	Complications	Maintenance
	years	mil-lions	per cent	grams	per cent normal			
25	58	5.12	103	3.83	105	—	—	E
	58	2.19	59	3.27	49	—	—	E
26	51	4.59	86	3.14	97	—	+	D
	50	4.22	67	2.73	72	—	+	D
27	57	4.4	107	4.69	75	—	+	D
28	82	5.03	81	2.00	42*	+++	++	E
		3.82	75	1.69	29*	+++	++	E
29	63	4.46	94	7.82	70	+++	++	?
30	63	3.64	82	5.41	65	+++	++	D
31	66	3.42	75	1.52	33	+++	+++	D
32	62	3.72	86	3.57	89	—	—	D
33	60	4.11	88	5.40	59	—	—	D
34	63	2.85	76	4.08	60	+++	—	E
35	64	3.83	77	3.79	83	+++	+	D
	64	1.91	53	2.79	54	+++	+	D
36		3.31	66	2.97	63	+++	+	?
37	57	3.30	64	4.22	68	+++	—	?
	57	1.06	31	3.57	41	+++	—	?
38	40	2.25	57	7.78	110	—	—	D
	42	3.97	84	2.55	79	—	—	D
39	62	1.64	44	3.54	39	+++	+	D
	62	2.80	79	2.70	44	+++	+	?
40	58	1.52	44	3.53	75	+++	+	?
41	41	1.87	46	3.29	58	+++	—	?
42	72	2.10	64	2.94	39	+	+	?
43	67	2.12	66	4.40	84	+	+	?
44	64	2.62	87	3.89	89	—	—	D
45	51	2.36	75	3.41	51	+	—	?
46	64	2.41	70	4.30	57	+	—	D
	64	4.72	97	4.49	50	+	—	D
47	61	2.12	59	3.77	89	+	—	E
48	51	5.27	109	4.19	123	—	+	E
49	56	5.40	107	2.56	127	—	+	D
50	30	4.79	96	1.74	72	—	—	D
	31	4.34	92	1.65	129	—	—	D
51	72	1.24	28	1.18	28	++	++	D

of xylose. The average of the urea clearances of these individuals was 115.2 per cent. These xylose excretions are slightly lower than those reported by Dominguez and Pomerene (18) whose 3 normal individuals who received 25 grams of xylose excreted from 4.86 to 7.66 grams in 5 hours. Higher results, however, might be expected from the methods used by them.

The average excretion during the 71 examinations on 48 patients having pernicious anemia was 4.28 grams. The average urea clearance, however, was only 78 per cent.

In addition to the patients typical of pernicious anemia, 3 other patients (Cases 49, 50, and 51) were examined. Before treatment, these patients had blood pictures similar to those seen in pernicious anemia, but had hydrochloric acid in their gastric contents. Two of these patients can be

classified as having non-tropical sprue. They both require liver extract by injection to maintain a normal red blood cell count. In both of these the urea clearances were normal but the secretion of xylose was markedly decreased. Case 49, with urea clearance of 127 per cent, excreted only 2.56 grams while Case 50 excreted 1.74 and 1.65 grams during two separate 5 hour periods when his urea clearances were 72 and 129 per cent of normal, respectively. Examination of Figure 1, Case 50, shows that there was only a very slight rise in blood xylose during the second test. It is obvious that in these two patients there was a decreased absorption of xylose. Case 51 also requires liver extract by injection. This patient excreted the least xylose in the series, yet there was little xylose in the blood after 5 hours, although the urea clearance was only 28 per cent of normal. The findings in these patients who, without doubt, have disturbances of absorption seemed to indicate that the simultaneous determination of blood and urine xylose following xylose ingestion together with measurements of urea clearance offers a method of measuring absorption from the gastro-intestinal tract.

TABLE II

*Average excretion of xylose in pernicious anemia patients grouped as to their urea clearance*

Urea clearance	Number of patients	Number of examinations	Average xylose excretion 5 hours	Below 4.26 grams	Over 4.26 grams
			grams		
Below 50 per cent of normal...	9	14	2.89	9	0
50 to 69 per cent of normal....	15	20	3.77	11	4
70 to 89 per cent of normal....	19	21	4.22	12	7
90 to 173 per cent of normal...	13	15	5.48	3	10
70 to 173 per cent of normal...	28	36	4.72	12	16
Under 70 per cent of normal...	23	34	3.39	19	4

Table II shows that the kidney function markedly influenced the amount of xylose excreted. There was a progressive increase in average secretion of xylose with increases in the urea clearance. The 28 patients having urea clearances of 70 per cent or better excreted an average of 4.72 grams of xylose. This is slightly higher than the average for the normals. There was, however, much greater spread in the values than in the normals. In individual cases the xylose excretion did not necessarily parallel the urea clearance. Exami-

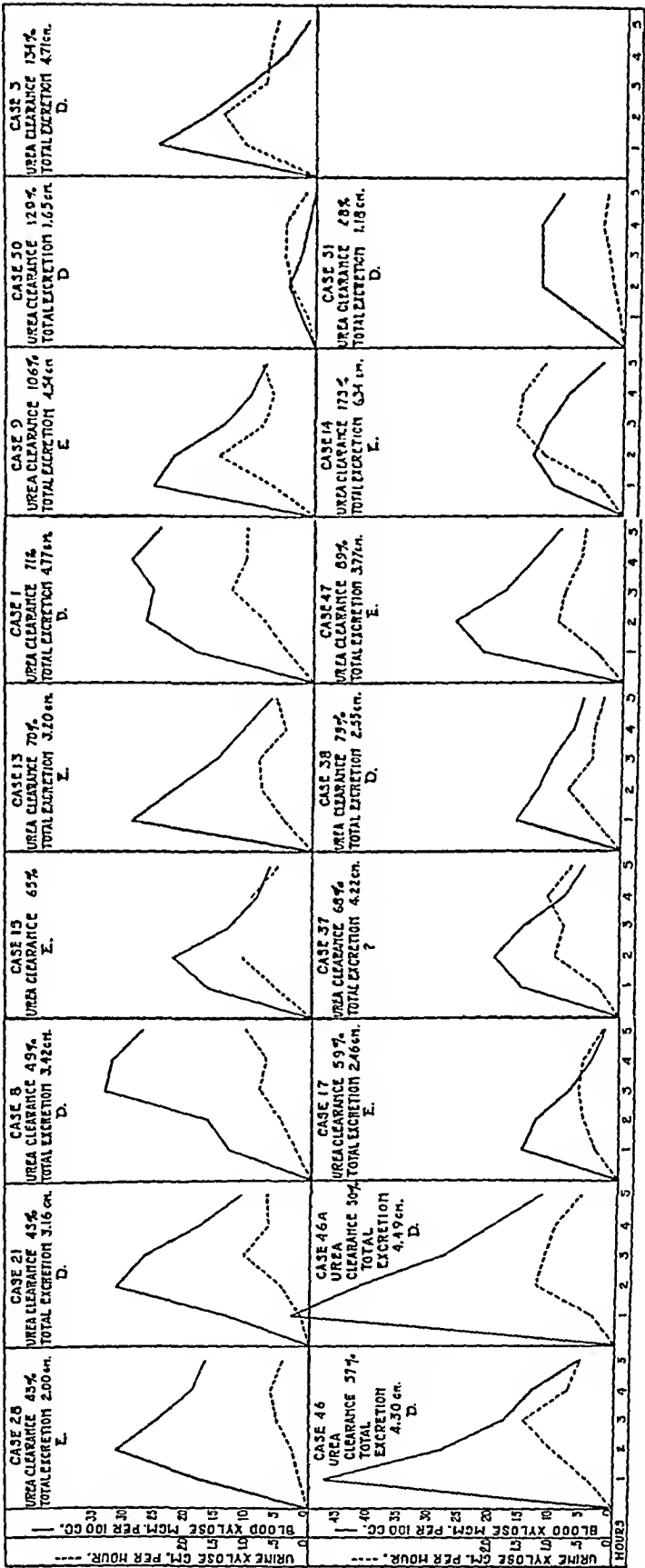


FIG. 1. SIMULTANEOUS BLOOD AND URINE XYLOSE CURVES IN 14 PATIENTS HAVING PERNICIOUS ANEMIA (15 EXAMINATIONS) AND IN 2 PATIENTS (50 AND 51) WHO HAVE HAD A MACROCYTIC HYPERCHROMIC ANEMIA BUT WHO HAVE FREE HCL IN THE GASTRIC CONTENTS  
E = easy to maintain normal red blood cell count on oral liver. D = difficult to maintain normal red blood cell count—i.e. require liver extract by injection.

TABLE III

*Average excretion of xylose in normal subjects and in various clinical groups of pernicious anemia patients*

	Num- ber of pa- tients	Num- ber of exami- nations	Aver- age xylose excre- tion 5 hours	Aver- age urea clear- ance	Below 4.26 grams	Over 4.26 grams
			grams	per cent normal		
1. Normals.....	8	8	4.68	115.2	0	8
I. Pernicious anemia patients.....	48	71	4.24	78.0	28	20
A. Red blood cell count not normal.....	27	31	3.76	63.7	21	6
B. Normal red blood cell count.....	31	39	4.49	84.4	15	16
1. Early involvement of central nervous system.....	20	24	4.99	94.8	6	14
2. Moderately advanced involvement of central nervous system.....	11	15	3.63	63.5	9	2
3. Under 60 years of age.....	17	21	4.54	93.0	8	9
4. 60 years or over.....	14	18	4.44	71.0	7	7
5. Having no known complications.....	13	17	4.98	98.5	4	9
6. Having complications—such as arteriosclerosis, infec- tions, etc.....	18	22	4.15	72.3	11	7
7. Difficult to maintain with normal red blood cell count ..	15	19	4.09	75.6	8	7
8. Easy to maintain with normal red blood cell count.....	15	19	4.59	91.3	7	8

nation of the simultaneous blood and urine curves (Figure 1) yields many interesting data. Cases 13, 17, 38, 50, and 51 all nearly completely cleared their blood of xylose in 5 hours, yet they excreted markedly decreased amounts of xylose. The fact that the final blood xylose was so low indicates to us that the excretory function of the kidney was not the limiting factor to the small amount of xylose excreted by the kidney. Case 1, however, excreted 4.77 grams in 5 hours, yet her ability to absorb was such that she still had high blood xylose at the end of 5 hours. The differences in the blood curves and the great variation in the amounts of xylose excreted by Cases 28, 21, 8, and 46a, who had only a difference of 7 per cent in urea clearances, likewise demonstrate the importance absorption plays in the excretion of orally administered xylose. The second examination on Case 38 was done during a slight blood relapse which occurred while taking the same amount of parenterally administered liver extract that had previously brought her blood to normal. In this patient there was a drop in urea clearance from 110 to 79 per cent, but this drop cannot account for the marked decrease in excretion of xylose. Thus, it is evident that absorption has a very definite influence on the amount of xylose excreted.

As is shown in Table III, the patients who had normal red blood cell counts were more apt to have higher xylose excretions than those who

had red blood cell counts below normal. However, the average urea clearance in the patients having normal red blood cell counts was 84.4 per cent, while the patients not having a normal red blood cell count had an average urea clearance of only 63.7 per cent. In many of the patients there was a marked increase in urea clearance following an induced remission, and this apparently accounts for the greater excretion of xylose in the patients having normal red blood cell counts. Since the low urea clearance on patients in blood relapse is apt to be temporary and since the kidney function so markedly influences the excretion of xylose, only patients having a normal red blood cell count were considered in making the comparisons in Table III.

The average excretion of xylose (see Table III) in patients having more advanced involvement of the central nervous system or advanced arteriosclerosis, infectious or degenerative complications was lower than in those patients not having these complications. However, the lower average excretion may be accounted for by the fact that these patients also had a lower average urea clearance. Of the 15 patients excreting less than 4.26 grams of xylose, only two did not have at least one of these complicating factors, while 7 of the 16 excreting more than 4.26 grams had no known complications. It cannot be said that increase in age in itself was associated with a decrease in excretion of xylose in these patients.



although there was a progressive decrease in urea clearance with increasing age.

There were 30 patients who had been followed for a sufficient length of time to be considered as to maintenance dosage of liver. As seen in Tables I and III and in Figure 1, there seems to be little if any correlation between the amounts of xylose excreted and the amount of liver extract required to maintain the blood at normal levels. In Cases 2, 6, 22, and 26 (see Table I) it was felt that the small amounts of xylose excreted in urine might indicate that they did not have satisfactory absorption from the gastrointestinal tract and therefore required larger amounts of liver extract. Cases 13, 16, 17, and 25, however, did not excrete xylose any better, but they have maintained their blood at normal levels for prolonged periods of time on oral liver therapy. In two of these latter patients (Cases 13 and 17), examination of the simultaneous blood and urine curves shows conclusively that the decreased excretion was limited by the absorption of xylose and not by the excretory ability of the kidneys. Cases 1, 4, 5, 12, 27, and 46, who have had one or more severe relapses while taking potent material by mouth in customary dosage, certainly cannot be said to have any difficulty in absorbing xylose from the gastrointestinal tract.

#### SUMMARY

The results of these studies indicate that absorption from the gastro-intestinal tract greatly influences the amount of orally administered xylose excreted in the urine during the first 5 hours after its ingestion. Thus it would appear that study of the urinary and blood xylose following its oral administration yields considerable information as to the absorptive ability of the gastrointestinal tract. If the kidney function is normal as shown by the urea clearance test and the excretion of xylose is low, it can be assumed that absorption of xylose from the gastro-intestinal tract is poor. This assumption is justified since it has been shown that xylose is not metabolized, passes through the liver unchanged, and can be recovered from the urine. Even in patients having lowered kidney function, information may be gained as to their ability to absorb xylose from the gastro-intestinal tract if, in addition to deter-

mining the xylose in urine, blood xylose curves are made. It has been shown that the xylose disappears from the blood stream at a rate proportional to the actual momentary concentration of the nonfermentable reducing substances in the blood and that there is a retention of xylose in the blood if kidney function is poor. Therefore, if the kidney function is decreased and the blood xylose does not rise as expected and is not retained in blood at 5 hours, it would appear that the decreased xylose excretion is due in part, at least, to a decrease in absorption from the gastrointestinal tract.

In these studies we were unable to demonstrate any constant abnormality in absorption of xylose in pernicious anemia patients. The average excretion of the pernicious anemia patients having normal urea clearance was slightly higher than that of the normal individuals. There was no correlation between the amounts of xylose absorbed by patients having pernicious anemia and the amounts of orally administered liver extract required to maintain the patients with normal blood counts. The 2 patients clinically diagnosed as non-tropical sprue and another patient having free acid in the gastric juice but having a blood picture of pernicious anemia had markedly decreased absorption of xylose from the gastrointestinal tract in the absence of diarrhea. All three of these patients require liver extract by injection to maintain a normal red blood cell count.

Since there may be a marked difference in the molecular size of xylose and that of the active principle of the liver, it does not necessarily follow that patients able to absorb xylose will absorb the active principle satisfactorily. We believe, however, that the results obtained from these studies indicate that differences in absorption from the gastro-intestinal tract can be demonstrated in pernicious anemia patients, although these differences seem to have little or no relationship to the amount of orally administered liver extract required by the patient to maintain a normal red blood cell count.

#### CONCLUSIONS

No consistent abnormality in absorption of xylose from the gastro-intestinal tract could be demonstrated in patients having pernicious anemia.

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# THE SIGNIFICANCE OF THE SODIUM AND POTASSIUM CONTENT OF MUSCLE TISSUE AND THE RELATION OF THE AMOUNT OF EDEMA FLUID IN MUSCLE TO THE LEVEL OF SERUM PROTEIN IN EXPERIMENTAL NUTRITIONAL EDEMA

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In investigating the mechanism of edema formation, we have made a study of the relations of sodium and potassium to the water in muscles of animals suffering from nutritional edema, in order to learn whether these relations provide evidence as to the validity of the hypothesis that fluid retention associated with hypoproteinemia is simply an accumulation of an ultrafiltrate from the circulating plasma. These relations might also indicate whether sodium has any rôle in the production of edema other than that of being the chief base of plasma filtrate, this question having been often raised by the observed necessity of ingested sodium for the formation of experimental plasmapheresis edemas in dogs (1, 2, 3), and for the occurrence of nutritional (4) and nephrotic (5) edemas in man. A nutritional edema in white rats resulting from a protein-poor carrot diet (6, 7) and shown to be accompanied by low serum proteins (8, 9) was decided upon as a practical experimental setting for the problem.

## DIETS

Kohman (6) demonstrated that the edema in her animals had not resulted from the lack of any of the vitamins then known, and was presumably the result of protein deficiency in the diet. Vitamin G had not then been identified and may not have been supplied in sufficient quantity to Kohman's rats by the wheat-germ extract. We have sought to learn whether such deficiency could have had any effect on the course of the edema. Accordingly, a group of five animals was placed on the Kohman wet diet to which was added daily, in a separate dish, 0.05 gram of a yeast concentrate powder.<sup>1</sup> This group of animals in Table IV is numbered from  $K + B_{21}$  to  $K + B_{25}$ , and as controls on this group there were five animals,  $K + YP_1$  to  $K + YP_5$ , which were given the Kohman diet with addition, in a separate dish, of 0.05 gram of the yeast protein remaining from

the concentrate powder after extraction of the B-complex. There were also two other control groups:  $K + CP_1$  to  $K + CP_4$ , Kohman diet plus 0.05 grams of casein extracted free of B-complex given in a separate dish daily; and  $K + G_2$ ,  $K + G_4$ , Kohman diet plus the equivalent amount of yeast concentrate which had first been autoclaved to destroy completely its vitamin  $B_1$  content only,<sup>2</sup> given in a separate dish daily. Thus all four of these groups did receive daily a very small amount of protein with or without vitamin G. In addition, vitamin  $B_1$  was supplied in the form of a solution of the pure crystals (Merck) rather than by adding wheat-germ extract to the carrot diet. All of these small servings of protein with  $B_1$  solution added, were, with few exceptions, completely consumed daily by the rats. The fact that these animals are all included in the second series of edematous rats (Table IV) records the result that they all showed retention of fluid in varying degree. Though there was a little delay, as compared to the group on the unmodified Kohman wet diet ( $K_{21}$  to  $K_{25}$ ), in the rapidity with which these animals lost weight from malnutrition and with which they accumulated fluid, the general trend was the same as the  $K$  group, and there were no significant differences among the various groups outlined above. It was obvious then, that addition each day to the diet of 0.05 gram protein, whether this contained vitamin G or not, was sufficient to defer slightly in some of the animals the effects of the unmodified Kohman diet. The individual animal that showed the greatest delay in becoming edematous can be seen from Table IV to be  $K + CP_1$ , which after 148 days of diet (the longest period) was still one of the least edematous animals of the whole group. The control normal group (Table III) had the Kohman wet diet with casein, extracted free of vitamin B-complex, replacing cornstarch in the amount of 20 per cent of the dry weight of the mixture. Three of the control animals ( $K + C + B_1$  to  $K + C + B_3$ ) had in addition 0.05 gram of the yeast concentrate added in a separate dish.<sup>3</sup>

The first series of protein-starved animals,  $K_3$  to  $K_{16}$ , Table II, were all given their vitamin  $B_1$  in wheat-germ

<sup>2</sup> Assayed by Dr. Siegfried Maurer and found to contain a concentration of vitamin G five times that of yeast.

<sup>3</sup> Three litters of rats (27 in all) comprised all of these groups. There were representatives of each litter in each group.

<sup>1</sup> This material was supplied by Mead Johnson and Company, and assayed by them to contain 80 units of vitamin  $B_1$  and 35 units of vitamin G per gram.

extract. The first series of normal animals, Table I, was composed of eight animals ( $N_2$  to  $N_{15}$ ) on a varied, high vitamin diet (wheat germ, brewer's yeast, lettuce, bacon, cheese, and a paste of casein, starch, minerals, lard, butter, and cod-liver oil); and a group of four animals ( $K + C_1$  to  $K + C_4$ ) on the Kohman wet diet with commercial casein replacing cornstarch in the amount of 15 per cent of the dry weight of the diet; and finally a group of four ( $K + A_1$  to  $K + A_4$ ) on Kohman wet diet with egg albumin, extracted free of the B-complex, replacing cornstarch in the amount of 15 per cent of the dry weight.

In all of these diets, water was allowed ad libitum, though very little seemed to be taken by the rats, the fresh carrots satisfying their needs for water. The animals in all groups had beginning weights ranging from 68 grams to 105 grams. The youngest litter of known age was 49 days old when placed on the diets and the oldest was 69 days.

During the course of the protein-poor diets the weight curves showed a steady decline with small irregularities, and, after 9 or 10 weeks, occasional cycles of rapid gains and losses with some terminal rises due to sudden increases in fluid retention, as reported by others (6, 8). There is no need to show all of these curves here. There was seldom manifest edema or anasarca, though some had excessive pleural and peritoneal fluids when sacrificed (see Tables II and IV). The weight curves of the rats on normal and normal control diets showed satisfactory though not always maximal growths for these animals in all except the  $K + A$  group, where the gains in weight were small and growth could hardly be called satisfactory. (In this connection see footnote 6 on page 358.)

#### CHEMICAL DETERMINATIONS

The first series of determinations are recorded for normal animals in Table I and for animals with various amounts of fluid retention in Table II. The determinations include: total serum nitrogen, muscle water, muscle protein nitrogen, muscle sodium and muscle potassium. The ratio, protein-N: potassium for muscle is shown, and the concentration of sodium plus potassium in millimoles per liter of total muscle water is given. There are also recorded figures for amounts of intracellular and extracellular water per kilogram of whole tissue and the concentration of potassium in intracellular water, derived by calculation, with certain assumptions which will be discussed later.

The second series of determinations are recorded for normal rats in Table III and for rats with varying degrees of edema in Table IV. These determinations include: *A.* For serum; total nitrogen, albumin nitrogen, and by difference, globulin nitrogen. *B.* For muscle; per-

centage of water in whole wet tissue, percentage of water in fat-free tissue,<sup>4</sup> sodium and potassium in terms of fat-free wet tissue. The concentration of sodium plus potassium in millimoles per liter of total muscle water is also shown; and again, by calculations discussed later, the amounts of intracellular and extracellular water per kilogram of fat-free wet tissue and the concentration of potassium in intracellular water.

In Tables II and IV are also included determinations of sodium in six abdominal or chest fluids from edematous rats as indicated and in one subcutaneous fluid from a blister on the chest of rat  $K + B_{24}$ . The fluids found in seven more animals in these two series were either somewhat bloody or were obtained in too small an amount to make possible a sodium determination with the usual accuracy.

#### METHODS

The animals included in Tables I and II were sacrificed by a blow on the head. Because this often caused loss of a good deal of blood with the consequence that it was difficult to get blood from the heart in satisfactory amounts, the later groups, Tables III and IV, were sacrificed by piercing the medulla oblongata with a needle. From most of these latter enough blood was secured from the hearts to make possible determinations of serum albumin in addition to total serum proteins.

If there was any chest or abdominal fluid it was taken with a capillary pipette and transferred into a centrifuge tube. The blood was then taken from the heart by capillary pipette.

The animal was next placed in a closed chamber in which the atmosphere had been saturated with water vapor from a pan of water, and by inserting the hands through rubber tubes (sections of tire inner tubes attached to round openings in the chamber) the skinning of the legs, cutting off of the muscles, and placing them into weighing bottles could all be carried out within the chamber by vision through its glass top. This "humidor" was designed to prevent evaporation from the muscle during the handling. The weighing bottles having been previously weighed empty from a desiccator were allowed to come to temperature equilibrium in the "humidor" before being opened, so that no appreciable moisture would settle on the glass inside the bottles when opened. The tightly stoppered bottles containing the wet muscle were again brought to equilibrium in a desiccator for weighing the wet muscle. The tissue was then dried in an oven at 105° C. Those included in Tables I and II were brought to constant weight in the milligram place, the larger samples (normals) re-

<sup>4</sup>Fat extraction was suggested to us by Prof. A. B. Hastings.

quiring as much as 19 days. Those in Tables III and IV, which were later to be extracted to remove fat, were dried to constant weight within 0.3 mgm., which required up to 35 days for samples up to 14 grams wet weight. It was hoped to eliminate moisture so completely from these samples that the later extraction with dry ethyl ether and petroleum would remove no salts. However, it was found in some preliminary experiments that from 2 to 5 per cent of the total amounts of sodium and potassium of the samples was removed during the extraction, whether because of moisture in the sample or in the ethyl ether, or whether because very minute scales of dried muscle tissue were sometimes lifted out with the capillary pipettes used in removing the extracting fluid. The last possibility hardly seems an adequate explanation of the transfer of so large an amount of base. At any rate, it was decided to evaporate the extracts in the quartz beakers in which the muscles were later to be ashed and thus, although a fat-free weight was obtained on the dry muscle for calculation, the extracted fats including whatever base was removed with them were ashed along with the fat-free tissue. This discussion obviously applies only to the determinations included in Tables III and IV since those included in Tables I and II were not made on fat-free tissues. The fat extractions were carried out according to the technique of Hastings and Eichelberger (10). The ashing of tissue in quartz with sulfuric acid has been described (22, 28).

The muscles analyzed in Tables I and II were not ashed in the same way, since determinations of muscle protein were made on these samples, and the bases had to be quantitatively extracted out of the dried mass. This was accomplished as follows: The tissue was allowed to stand covered with water until thoroughly soaked. Then enough trichloroacetic acid was added to make a 20 per cent solution. After standing and macerating thoroughly in a mortar, the extract was filtered off and maceration was repeated with nine portions of 10 per cent trichloroacetic acid while a quantitative transfer to the filter was accomplished. The extract was subsequently treated for phosphate precipitation before being made up to known volume, after which aliquot portions were used for dry ashing in platinum crucibles. This method of extraction was suggested by the procedure described by Salit (11) for sodium determinations in tissues. Six preliminary ashings of beef and rat muscle fibers, extracted in this way, convinced us that the bases were quantitatively extracted, since the total ash obtained in platinum crucibles from the protein remaining from 5 to 9 gram samples of muscle weighed only from 1 to 2 mgm., and being for the most part insoluble could be accounted for by glass ground off from the mortar. This ash contained indeterminable traces of sodium and potassium.

The muscle fiber having been quantitatively collected on a filter paper was transferred with the filter to a large Kjeldahl flask and digested for macro-Kjeldahl determinations. Aliquot portions of the digest were used for

duplicate determinations which always agreed to 0.5 per cent.

Because of the variation in the amount of muscle fat and of the impracticability of mincing the material without loss of fluid, it was decided not to take two separate samples of muscle from one animal. There were two exceptions made. In Table I, analyses of two separate samples taken without mincing from Rat N<sub>9</sub> are shown, and in Table III, analyses on two samples from Rat K + C<sub>21</sub> are presented. However, for the normal animals (Tables I and III), where the samples were of sufficient size duplicate determinations were made as follows: for those in Table I, after the extraction of the minerals and the phosphate precipitation in the extract; for those in Table III, after the ashing. For the values in Tables I and III the duplicate sodium determinations agreed to within 3 per cent and the duplicate potassium determinations to within 1½ per cent. There was one exception to each of these standards; namely, the sodium determinations for K + C<sub>25</sub> were 7 per cent apart, and the potassium determinations for the second samples of K + C<sub>21</sub> were 14 per cent apart. In each of these two instances the single value that seemed the more reasonable is given in the Table and used for subsequent calculations. We obtained theoretical recoveries of sodium and potassium from known mixtures of NaCl, K<sub>2</sub>SO<sub>4</sub>, and H<sub>3</sub>PO<sub>4</sub> when put through separate phosphate precipitations before the ashings in platinum, or when put through separate ashings in quartz. Sodium and potassium determinations on muscle extracts in which the phosphate precipitation was done in duplicate agreed to 3 per cent and 1 per cent, respectively. Known amounts of these bases added to the extracts were recovered quantitatively. Duplicate ashings, in quartz, of dried ground homogeneous beef samples yielded sodium and potassium determinations which agreed to 1½ per cent.

The samples from the undernourished edematous animals and from the K + A group of Table I were so small that the material could not be divided for the duplication of even the final steps in the sodium and potassium determinations. In the case of protein nitrogen, duplicate determinations were made just as for normal animals. The single determinations in Tables II and IV ought to be dependable, granting always a possibility of some bad error committed unconsciously, which would bring a determination far out of line.

The final chemical methods used were the following.

1. *Removal of phosphate.* (a) In trichloroacetic acid extracts from muscles listed in Tables I and II (first series), the method of phosphate removal was based on that given by Hoffman (12) in preparation for total base determinations in urine. It was modified to the extent that the solutions were not transferred to volumetric flasks until after the precipitate of ferric ammonium acetate was well coagulated and all evolution of gases had ceased. Then, aliquot portions of filtrate were evaporated down with 3 cc. of 1:1 H<sub>2</sub>SO<sub>4</sub> in platinum crucibles for dry ashing. The ashing was

TABLE I  
Normal rats—first series

Number of rat	Diet period	Serum total nitrogen per 100 cc. $\times 6.25$	Muscle								
			Water of whole tissue	Protein nitrogen	Sodium	Potas- sium	Extracellular fluid	Intracellular water	Potas- sium	Sodium + potas- sium	Protein nitrogen potassium
	days		per cent	mM. per kgm. of whole tissue			ml. per kgm. of whole tissue		mM. per liter cell water	mM. per liter total H <sub>2</sub> O	ratio
ON VARIED NORMAL DIET											
N <sub>8</sub>	62	7.1	73.0	2130	21.6	97.7	151	580.5	167	163	21.8
N <sub>3</sub>	75	7.2	72.5	2162	22.0	97.2	153.5	573	168.5	164	22.25
N <sub>2</sub>	75	8.15	73.6	2162	21.4	98.5	149	588	166.5	163	22.0
N <sub>6</sub>	77	6.65	73.7	2164	20.15	102.0	140	598	169.5	166	21.2
N <sub>13</sub>	75	7.1	73.7	2227	22.9	101.7	160	579	174.5	169	21.8
N <sub>11</sub>	76	7.35	71.9	2095	20.9	97.8	146	574	169	165	21.4
N <sub>15</sub>	82	8.4	73.25	2210	23.8	100.7	166	568	176	170	21.9
N <sub>9</sub> *	93	8.05	72.2	2132	19.8	97.8	138	585	166	163	21.8
			73.1	2135	20.75	101.7	145	587	172	167.5	21.0
Range							166-138	568-598	176-166	170-163	
Average		7.50	73.0	2157	21.5	99.5	150	581	170	165.6	21.7
ON CARROT CONTROL DIET—KOHMAN DIET + CASEIN											
K+C <sub>4</sub>	79	6.6	73.25	2134	22.1	99.8	154	579.5	171	166.5	21.4
K+C <sub>1</sub>	82	6.9	74.2	2095	20.7	105.6	144.5	599	175	170	19.8
K+C <sub>2</sub>	86	7.5	73.85	2204	19.8	103.5	138	601.5	171	167	21.3
K+C <sub>3</sub>	93	7.9	74.3	2201	24.3	99.5	169.5	575	172	167	22.1
Range							169-138	575-601	175-171	170-166	
Average		7.2	73.9	2158	21.7	102.1	151.5	589	172	167.5	21.15
ON CARROT CONTROL DIET—KOHMAN DIET + EGG ALBUMIN†											
K+A <sub>4</sub>	82	6.5	75.0	2173	26.5	102.0	185	567	178.5	171	21.3
K+A <sub>3</sub>	103	5.05	74.8	2100	24.7	103.2	172	577	177.5	171	20.3
K+A <sub>2</sub>	126	6.35	75.0	2150	27.15	97.9	189.5	562.5	172.5	167	22.0
K+A <sub>1</sub>	131	6.5	74.2	2190	21.1	105.7	147	596	176	171	20.7
Range							189-147	562-596	178-172	171-167	
Average		6.1	74.8	2153	24.9	102.2	173.5	575.5	176	170	21.1
Complete range							189-138	562-601	178-166	171-163	
Complete average		7.1	73.5	2157	22.3	100.7	156	581	172	167.1	21.4

\* Two samples.

† See footnote 6 on page 358.

done in duplicate for the large samples but not for the small.

(b) In the aliquots of ash used for sodium determinations on the muscles listed in Tables III and IV (second series), the method of phosphate removal described by Butler and Tuthill (13) was used.

2. *Sodium.* The solutions for sodium determinations were evaporated to dryness in 30 cc. beakers, and the procedure of Butler and Tuthill (13) was followed.

Barber and Kolthoff (14) have stated that a contaminating precipitation of the potassium triple salt occurs only in the presence of more than 50 mgm. of KCl per cc., while a precipitation of some  $K_2SO_4$  itself may occur in the presence of somewhat smaller amounts of  $K_2SO_4$ . Butler and Tuthill have accepted this finding. Nevertheless, we found that sodium precipitates obtained from the muscles (either series) and also from known mixtures of  $K_2SO_4$  and NaCl of approximately muscle propor-

tions contained some potassium. Mond and Netter (15) also found sodium determinations on muscle tissues to be higher than their true values when made as uranyl-zinc-sodium-acetate precipitates in the presence of the muscle potassium. We therefore worked out a correction curve for the increase in weight of sodium precipitate with increasing amounts of potassium present, making due allowance for sodium blanks on the potassium sulfate used in the known mixtures. From this curve was taken the absolute weight increment to be subtracted from any given weight of sodium precipitate, on the basis of the amount of potassium known to be present in the given solution from the potassium value determined on another aliquot of the same ash. These corrections ran from 0 to 3.1 mgm. for the presence of 6 to 20 mgm. of potassium in the 2 cc. of solution used for sodium determination. It was only in the cases of the larger amounts of potassium that the correction was of an order of magni-

TABLE II  
*Edematous rats—first series*  
(Listed in order of increasing water content in whole tissue)

Number of rat	Diet period	Serum total nitrogen per 100 cc. X 6.25	Terminal weight changes	Free fluid found	Fluid sodium	Muscle								Protein nitrogen potassium
						Water of whole tissue	Protein nitrogen	Sodium	Potassium	Extra-cellular fluid	Intra-cellular water	Potassium	Sodium + potassium	
	days				mM. per liter	per cent	mM. per kgm. whole tissue			ml. per kgm. whole tissue		mM. per liter cell water	mM. per liter total H <sub>2</sub> O	ratio
K <sub>1</sub>	62	5.1	Gained 3 grams in 2 days	None	—	73.9	2073	29.8	91.5	208	533	170	164	22.6
K <sub>11</sub>	82	4.8	No gain	None	—	75.8	2120	27.8	100.3	194	566	176	169	21.15
K <sub>12</sub>	80	3.6	No gain	None	—	75.9	2191	33.7	97.3	235	526	183	172.5	22.5
K <sub>13</sub>	83	3.65	Gained 1½ grams in 2 days	Chest and abdom.* 0.3 + ml.	—	76.9	2026	34.1	90.8	238	533	168.5	162	22.3
K <sub>5</sub>	73	4.4	Gained 5 grams in 3 days	None	—	77.0	1936	34.8	90.7	243	529	169.5	163	21.3
K <sub>10</sub>	93	3.6	Gained 2 grams in 1 day. Other gains and losses	Chest and abdom. 0.5 + ml.	141.8	77.0	1996	36.3	89.6	253	519	171	163.5	22.3
K <sub>12</sub>	76	3.25	Gained 1½ grams in 1 day. Maintained 1 day	Abdom. only 0.4 + ml.	—	77.7	2026	28.8	99.5	201	578	171	165	20.4
K <sub>3</sub>	74	4.05	Earlier rapid gains and losses	None	—	78.1	1986	49.3	71.5	344	440	159†	154†	27.8
K <sub>9</sub>	92	3.0	Earlier rapid gains and losses	None	—	79.0	1860	51.1	82.7	356.5	437	186	169	22.5
K <sub>4</sub>	75	3.1	Gained 3 grams in 3 days	Chest and abdom. 1.8 + ml.	141.8	79.8	1795	53.1	73.9	370.5	431	168	159	24.3
K <sub>8</sub>	77	3.05	Gained 5 grams in 3 days. Maintained 1 day	Abdom. only 0.8 + ml.	149.1	80.0	1780	56.0	73.6	391	413	174	162	24.2
K <sub>14</sub>	75	2.75	Gained 4 grams in 1 day	Chest and abdom. 2.0 + ml.	144.8	80.5	1726	54.6	77.7	381	428	178	164	22.2
Range												186-165†	172-159†	
Average												174†	165†	

\* abdom. = abdominal.

† From the figures of both range and average the values for K<sub>3</sub> have been omitted—see discussion.



TABLE III  
Normal rats—second series

Number of rat	Diet period	Serum nitrogen × 6.25 per 100 cc.			Muscle							
		Total	Albumin	Globulin	Water		Sodium	Potassium	Extracellular fluid	Intracellular water	Potassium	Sodium + potassium
					Of whole tissue	Of fat free						
	days				per cent	per cent	m.eq. per kgm. of fat-free tissue		ml. per kgm. of fat-free tissue		mM. per liter cell water	mM. per liter total H <sub>2</sub> O
K+C+B <sub>2</sub>	84	8.5	4.15	4.35	72.65	77	25.5	93.1	178	595	155	154
K+C <sub>22</sub>	76	7.45	3.6	3.85	73.9	77.35	23.8	108.8	166	609	177.5	171
K+C+B <sub>1</sub>	147	7.65	4.45	3.2	74.4	76.9	21.95	97.4	153	617	157	155
K+C <sub>21</sub> *	147	7.3	3.7	3.6	75.35	76.65	19.25	98.0	134	633.5	154	153
					76.0	77.3	19.25	102.9	134	640	160	158
K+C+B <sub>4</sub>	144	7.45	3.75	3.7	71.3	76.65	27.2	99.2	190	578.5	170	165
K+C <sub>25</sub>	102	8.85	4.15	4.7	74.45	77.1	23.3	96.3	163	610	157	155
K+C <sub>24</sub>	144	7.5	3.7	3.8	74.0	Not completed						
Range									190-134	578-640	177-154	171-153
Average		7.8	3.9	3.9	74.0	77.0	22.9	99.4	160	612	161.5	158.7

\* Two samples.

tude equal to or larger than the experimental error of the sodium method. However, the proportionate correction was applied to all weights. The removal of potassium with saturated ammonium perchlorate solution proved to be much more tedious and to bring about results on known sodium solutions that were approximately 2 per cent low.

3. *Potassium.* The aliquots of ash for potassium determinations in both series were evaporated to dryness in 30 cc. beakers, and the potassium determinations were made by the Shohl and Bennett (16) method using their colorimetric procedure.

In edema fluids the protein was removed with 20 per cent trichloroacetic acid and for sodium determination an aliquot of the filtrate was transferred directly to the uranyl-zinc-acetate reagent in the glass filter.

The serum albumin and globulins were separated by the method of Howe (17). All serum nitrogen determinations were done by the Koch and McMeekin (18) micro-Kjeldahl method.

Blanks on all reagents used in all procedures were determined repeatedly.

### Discussion of tables

#### The normal rats

In Table I, the animals are not listed in a particular order. They are, however, grouped according to diet, and the litter mates are listed

in succession. In Table III, the second group of normals is arranged also with litter mates in succession, the two diets being differentiated by the numbers assigned to the animals. In comparing the two tables of normals it is to be noted that the average percentage of water in the whole muscle of rats in Table III agrees very closely to that found for the previous K + C group, that the N group is about 1 per cent lower and the K + A group about 1 per cent higher.<sup>5</sup> The maximum variation in water among the animals in any diet unit in Table I, however, is 2 per cent, while the variation in Table III reaches almost 5 per cent in the water figures. It is interesting to see that the percentage of water based on fat-free tissue has greater constancy than the percentage of water of whole tissue. The variation between extreme values in fat-free tissues does not exceed 0.7 per cent. This extreme variation of the whole group is shown between the two samples from one animal, K + C<sub>21</sub>. The average for fat-free tissue is 3 per cent higher than the average for whole tissue. The 5 per cent varia-

<sup>5</sup> See footnote 6 on page 358.

tion noted in this series in terms of whole tissue can, then, be explained chiefly as due to variations in fat content of the whole tissues.

stancy in sodium and in potassium figures in the fat-free tissue of the normal animals in Table III than in the corresponding figures based on whole tissue of the normal animals in Table I. One

TABLE IV  
*Edematous rats—second series*  
(Listed in order of increasing water content in fat-free tissue)

Number of rat	Diet period	Serum nitrogen × 6.25 per 100 cc.			Terminal weight changes	Free fluid found	Fluid sodium	Muscle							
		Total	Albumin	Globulin				Water		Sodium	Potassium	Extra-cellular fluid	Intra-cellular water	Potassium	Sodium + potassium
								Of whole tissue	Of fat free						
	days						mM. per liter	per cent	per cent	mM. per kgm. fat-free tissue		ml. per kgm. fat-free tissue		mM. per liter cell water	mM. per liter total H <sub>2</sub> O
K+B <sub>21</sub>	75	4.8	2.2	2.6	No gain	None	—	75.0	77.85	22.1	113.7	154	626	180.5	174
K+CP <sub>1</sub>	148	3.25	1.2	2.05	No gain	None	—	76.2	77.9	34.7	97.9	242	539	180	170
K+CP <sub>4</sub>	98	4.65	1.7	2.95	No gain	None	—	73.65	78.0	27.3	111.3	190.5	591.5	187	178
K+YP <sub>3</sub>	84	4.35	1.95	2.4	Gained 1½ grams 1 day after 1 gram loss	None	—	73.7	78.0	26.7	108.3	186.5	595.5	180.5	173
K+CP <sub>2</sub>	76	5.35	2.35	3.0	Gained 3 grams 2 days after 4 grams loss	None	—	72.15	78.1	23.55	109.2	164	618	176	170
K+B <sub>21</sub>	83	4.7	1.95	2.75	Gained 2 grams in 2 days	None	—	74.1	78.4	24.8	104.0	173	613	168.5	164
K+CP <sub>3</sub>	144	3.1	1.1	2.0	Gain and loss 2 grams in 2 days. Earlier cycles also	Chest and abdom. 0.3 + ml.	—	77.2	78.6	46.8	83.3	327	462	177.5	165.5
K+G <sub>2</sub>	75	5.2	2.5	2.7	No gain	None	—	76.9	78.8	29.0	108.8	202	588	183.5	175
K <sub>21</sub>	83	4.65	2.05	2.6	Gained 1½ grams 1 day after 3 grams loss	None	—	75.1	78.9	27.8	104.3	194	597	173	167
K+YP <sub>1</sub>	76	6.2	2.5	3.7	No gain	None	—	75.8	79.0	25.25	106.7	176	616	172	167
K+B <sub>21</sub>	104	3.45	1.1	2.35	No gain	None	—	76.25	79.0	30.0	102.5	209	583	174.5	168
K <sub>21</sub>	104	2.85	0.8	2.05	No gain	Abdom. 0.3 + ml.	—	76.95	79.35	33.45	101.8	233	562.5	179	171
K <sub>21</sub>	105	3.55	1.25	2.3	No gain	None	—	71.2	79.4	35.3	89.45	246	550	161	157
K+YP <sub>4</sub>	98	4.0	1.6	2.4	No gain	None	—	77.0	79.5	36.9	98.4	257.5	540	180.5	170
K <sub>21</sub>	75	3.05	—	—	No gain	Chest and abdom. 0.2 + ml.	—	78.55	80.4	50.3	84.6	351	456	182.5	168
K+G <sub>4</sub>	98	3.1	1.3	1.8	No gain	None	—	76.2	80.5	38.5	94.8	269	539	174	166
K+B <sub>21</sub>	144	3.65	—	—	No gain	Chest and abdom. 0.4 + ml.	—	80.7	81.2	43.95	73.7	307	508	143*	145*
K+YP <sub>4</sub>	138	4.4	—	—	Gained 2½ grams in 1 day	Chest and abdom. 1.5 + ml., subcu. 0.2 + ml.	140.5	81.25	81.7	61.65	68.7	430	390	172	159.5
K+B <sub>21</sub>	138	2.45	—	—	Gradual gain of 7 grams in 15 days, 2 grams in last 2 days	Chest and abdom. 1.0 + ml., subcu. 0.5 + ml.	141.3 143.9	81.55	83.25	59.0	67.25	411	425.5	154	152
K <sub>21</sub>	125	2.65	1.0	1.65	Lost 4½ grams 1 day after gain of 5 grams in 3 days. Two other cycles	Chest only 0.4 + ml.	—	81.6	83.3	70.25	63.2	490	348	176	160
Range														167-154*	178-152*
Average														175.5*	166.9*

\* From the figures of both range and average the values for K+B<sub>21</sub> have been omitted since it seems not unlikely that their extreme lowness is due to technical error.

might have expected that these bases, which are presumed to be in solution in the muscle water, would follow water in showing less variation when based on fat-free tissue. The variation in potassium of this fat-free series is, in fact, greater than that in the other series and taken by itself the N group of animals, made up of a variety of litters but all on the same non-carrot normal diet, shows the greatest constancy in sodium and potassium figures even in terms of tissue which must have had some variations in fat content. From this it might be inferred that the animals on the control carrot diets, and especially those of the second series, had larger variations in the relative amounts of their intracellular and extracellular muscle water than the N series. The possibility of less accuracy in the method of ashing in quartz, used preparatory to these determinations in the second series, was considered, but from the ashings of known salts we could get no evidence of less reliability in this procedure than in our previous procedure of extraction plus ashing in platinum. The final chemical methods used in both series were the same.

The serum albumin and globulin figures recorded for normal rats in Table III indicate that the serum albumin:globulin ratio in white rats is 1 as contrasted with about 2 for this ratio in man.

#### *The edematous rats*

In Table II the animals are listed in the order of increasing water content in whole tissue, in Table IV in the order of increasing water content of fat-free wet tissue. There is a very rough correlation of increasing water contents with decreasing serum total nitrogen and serum albumin figures. This correlation which is better when calculated extracellular fluid contents are compared with serum protein values will be discussed below.

#### *Calculations*

The calculation of extracellular fluid was based on the assumptions that all the sodium of the muscle is in the extracellular fluid and that its concentration in this fluid is 143.3 mM. per liter of fluid, which is the mean of the seven values determined for the concentration of sodium in free fluids from edematous rats. The cell water

was calculated by difference between fluid water and total water (using 99 per cent of the fluid value as its water value). Finally, having made an allowance of 4 mM. of potassium per liter of extracellular fluid (this being the medium value for potassium from among those reported for various edema fluids by several authors (19, 20, 21)) the concentration of the remaining potassium in the cell water was calculated. Because of physiological variation of sodium concentration in extracellular fluid a mean value cannot be considered as accurate for any individual animal even if it is based on more determinations than we had. Since a constant value (143.3) was used for all our animals as a basis for the several calculations, the inaccuracy involved here must be reflected in an exaggerated variation between animals in the final calculated result; namely, the potassium concentration in the cell water.

The validity of the assumption that all muscle sodium is in extracellular fluid is discussed below. So also is the consideration that the concentration of sodium in this fluid should have a slightly different value in the fluid of the normal animal than that found in the edema fluids. Hastings and Eichelberger (10) and Harrison, Darrow and Yannet (22) base their calculations on the assumption that chloride is present only in extracellular fluid and on chloride concentrations found for each animal by applying the Donnan ratio to the individual serum chloride figures.

#### DISCUSSION

##### *Relation of edema fluid in muscle to serum protein levels*

In the figures for extracellular fluid content of muscle at different serum protein levels we have quantitative evidence that the muscle tissue becomes slightly edematous with the first decrease of serum protein, and that it becomes increasingly so with decreasing serum proteins.<sup>6</sup> This rela-

<sup>6</sup> The four animals, K + A<sub>1</sub> to K + A<sub>4</sub>, though they are included in Table I as normal animals, ought probably to be considered as on the borderline of fluid retention. Their diet was hardly adequate in protein for growth, and this is reflected in a tendency to lower serum protein as compared to other normals, and a tendency to slightly higher muscle sodium and water values. The average calculated extracellular water figure for this group is consequently somewhat higher than that of the other normal groups.

tionship is plotted in Figure 1. It shows that there are increasing slight accumulations of edema fluid in the muscle while the serum total protein values are dropping from 6.5 to 4.5, the serum albumin values from 3.5 to 2.0, and that the accumulation of fluid becomes progressively greater when the serum total protein falls below 4.5 per cent and the albumin below 2.0 per cent. Weech, Snelling and Goettsch (23) studied the behavior of weight curves and their relation to

The theory of a "critical level of plasma proteins" which must be reached before edema occurs has grown out of the circumstance that the presence of edema can usually be ascertained only by such gross methods as the following: body weight increase, palpability, swelling of parts and occurrence of free fluid in the cavities. Moreover, a completely rational filtration theory for the mechanism of edema formation must include the conception that filtration begins with the first

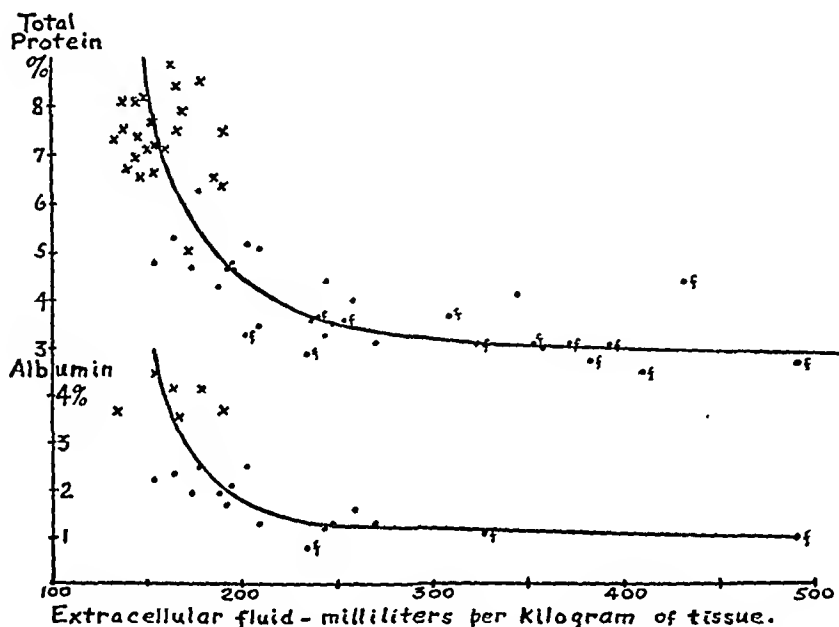


FIG. 1. THE RELATION OF THE EXTRACELLULAR FLUID CONTENT OF MUSCLE TO THE SERUM TOTAL PROTEIN AND ALBUMIN VALUES.

The crosses represent values for normal animals and the dots for edematous animals. The symbol f indicates that free fluid was found in these animals. The extracellular fluid values per kilogram of whole tissue (Tables I and II) are plotted on the same scale as the extracellular fluid per kilogram of fat-free tissue (Tables III and IV).

declining plasma proteins as well as sodium, chloride, and nitrogen balances in dogs with nutritional and plasmapheresis edemas. They conclude that there is no indication of "the existence of a 'margin of safety' in the plasma oncotic pressure which must be exceeded before retention of salt and water begins" and that their "data argue in favor of gradual fluid retention which seems to accompany the entire process of plasma protein depletion." It seems likely to us that this situation exists in the tissues during the course of plasma protein depletion in nephrosis, also.

decrease of the plasma oncotic pressure below the level of the balancing pressures.

#### *Relation of muscle protein nitrogen to potassium*

In the normal rats of Table I the ratio of muscle protein nitrogen to muscle potassium has extreme values as far apart as 11.4 per cent of the average ratio. It would seem then that as related to cell protein the variation in amounts of cell water, if this is determined by a constant concentration of potassium, can reach the extreme of about 12 per cent in normal tissues. If not,

the variability in the ratio must be a combination of variations in concentration of potassium in cell water and in the amounts of cell water as compared to cell protein. In giving this significance to the ratio of protein-nitrogen to potassium the small amount of potassium in extracellular water is disregarded. Gamble, Ross and Tisdall (24) used the concept of a constant relationship of cell protein to cell water, together with their findings of potassium and nitrogen excretion during starvation, as a basis for calculating intracellular water lost by protein metabolism, and, by difference, the amount of intracellular water lost due to reduction of cell volume. The latter, they consider, is roughly determined by the amount of glycogen depletion. Laviates, D'Esopo and Harrison (25) review Gamble's reasoning and point out that the possibilities of large variations in cell water without "change of nitrogen or glycogen or even potassium balance as the result of a change in the molar concentration of the body water as a whole through the addition or removal of water without base or a change in the concentration of sodium in interstitial fluids, which necessitates an exchange of water with the cells in order to restore osmotic equilibrium" make it unreasonable to assume that "cellular protein or glycogen is associated in such fixed proportion with water that the excretion of one inevitably entails the elimination of an equivalent amount of the other."

It was therefore not to be expected that in the course of progressing edema and starvation the ratio of protein-nitrogen to potassium should be maintained at a constant level, but with such a range of variation in normals it is perhaps not surprising that nine out of twelve of the rats in Table II did maintain a ratio of muscle-protein-nitrogen:potassium in the normal range or very near its upper limit. Among these nine were two of the most edematous animals in the group. The nine ratios average somewhat higher (21.9) than the normal average (21.4), indicating perhaps a tendency to some loss of cell water accompanied by potassium beyond that from protein metabolism or a tendency to lowered potassium concentration in the cell water. The three remaining quite edematous animals showed a high ratio of nitrogen to potassium, which means either a loss

of water and its base out of the cells beyond the loss by protein metabolism or else a dilution of potassium within cell water. The latter alternative is not consistent with the results in general from calculations of potassium concentration based on the intracellular water values, but in the case of  $K_3$  which has the highest ratio, it appears that there may be an unusual dilution of potassium. However, a gross error in the potassium determination of this one muscle could account for both the high ratio of its protein nitrogen to potassium and the low calculated potassium concentration.

#### *Osmolal concentrations of bases in the two compartments of tissue water*

We are indebted to Prof. A. B. Hastings for his aid in clarifying our concept of how the values of the various constituents of a tissue in terms of a unit weight of tissue are affected by addition of water to that tissue. In considering the amount of fluid retention in any tissue it is misleading to use the water percentage or grams of water per kilogram of the original (normal) tissue as a basis from which to calculate the retention in the edematous tissue. A muscle, for example, which had 750 cc. of water per kilogram to begin with has had 250 cc. of water added to each kilogram of original tissue to make it an edematous muscle containing 800 cc. of water per kilogram. In other words the fluid added is 25 per cent of the original weight of tissue rather than only 5 per cent. Or, more simply, from the solids standpoint the 25 per cent solids are diluted to 20 per cent solids which means a change to  $\frac{5}{4}$  of the original bulk or addition of water equal to  $\frac{1}{4}$  the original weight. In the new kilogram of tissue then, there is  $\frac{1000}{1250} \times 250 \text{ cc.} = 200 \text{ cc.}$  of the retained water and the equivalents of sodium and chloride ions retained with it. Now, if originally, all the sodium and chloride ions in the kilogram of tissue were in solution in 150 cc. of its water (extracellular portion) and  $\frac{1000}{1250}$  of the original amount of sodium and of chloride ions is present in the kilogram of new tissue, the new kilogram of muscle now contains sodium and chloride ions in amounts equivalent to  $120 + 200$  cc. of water if the sodium and chloride ions were

added in the same concentrations as those in the original extracellular water (plasma filtrate concentrations). Hence the quantity of sodium and of chloride ions has increased per kilogram of tissue from an amount equivalent to 150 cc. of water to an amount equivalent to 320 cc. of water. Each will have more than doubled in value, then, while the water figure has increased only by  $\frac{1}{2}$  of its original value. The increase in sodium and chloride values per kilogram of tissue will be related to the increase in water value as 170 is to 50 which would seem to make the added sodium and chloride ions 3.4 times as concentrated in the added water as they really are. In this fact lies the fallacy of Lepore's (26) conclusion that chloride found in the muscle was "stored hypertonically." As Peters (27, page 137) points out "no interpretation can be made without knowledge of the total osmolar concentration in the interstitial fluids at the beginning and at the end of each experiment," and for this knowledge more complete data are required. However, it is not necessary to assign the great variability and the large values of Lepore's "molarity of stored chloride" to "large technical errors" as is done by Peters. Such variability and such apparently tremendous excesses of chloride as compared to water excesses are entirely possible when the figures are treated in that way. In the instances of only 1 per cent increase of water content in a tissue the discrepancies of excess chloride compared to excess water figures explainable by the above can even be more than doubled if the average normal value for water (used as a basis for calculation) happens to be off by 1 per cent or more of what is really normal for that individual animal. There is enough variability in the water of muscles of normal animals when fat is not extracted so that an average value may easily be 1 to 2 per cent off for an individual specimen. Hence molarity figures for chloride in the neighborhood of 1.0, as several that occur in Lepore's report, which are 8 to 9 times plasma filtrate molarity, are explainable by combination of this circumstance with the fallacious handling of figures as outlined above.

Though the calculations of amounts of extracellular water, and by difference, amounts of

intracellular water, could be done with more assurance on the basis of chloride determinations than on the basis of sodium, since all experimental evidence seems to point to the conclusion that no chloride whatever is to be attributed to muscle cells (Peters (27, pages 132, 133)) yet the broad conclusion of other workers that probably also no sodium is to be found in skeletal muscle cells (Peters (27, pages 129, 130)), gives us this basis for using our sodium figures. With the findings of Hastings and Eichelberger (10) in mind that "approximately 5 mM. of sodium per kilo of tissue" in dog muscle is attributable to the muscle cells, and the following conclusion of Harrison, Darrow, and Yannet (22) in mind also, that, "except for the skeletal sodium and 10 per cent of the sodium of dog muscle, all of the sodium of the body can be accounted for in the same volume of extracellular water which would contain the body chloride," we admit the possibility that from 10 to 20 per cent of our sodium in normal muscles should be excluded from calculation as osmotically active extracellular sodium.<sup>7</sup> Harrison, Darrow and Yannet found practically no "extra sodium" in the separate muscle determinations in rabbits, however. No one has reported the relationship of the muscle sodium to muscle chloride for rats, and it is to be regretted that we do not have the necessary data to do so. However, with 15 per cent allowance for "extra sodium" our several normal<sup>8</sup> averages for extracellular fluid would be lowered to 127 ml. (N group), 129 ml. ( $K + C_1$  to  $K + C_4$ ), and 136 ml. (Table III). As they now stand the averages are 150, 151, and 160, respectively. All of the extracellular water values in normal rats, as they stand without correction for "extra sodium," come within the range of 13 to 20 per cent of the muscle weight. This is in agreement with the more or less general conclusion from a variety of investigations concerning the amount of interstitial fluid in muscle tissue (Peters (27, pages 128, 130, 134, 141)). With a

<sup>7</sup> For discussion of "extra sodium" see Peters (27, pages 139 to 142). As a result of many perfusion experiments with frog legs, Mond and Netter (15) come to the conclusion that there is sodium in an amount up to 30 mgm. per cent which is not balanced by chloride, bound in some way to the surface of the muscle fiber.

<sup>8</sup> Excluding the  $K + A$  group, see footnote 6 on page 358.

15 per cent allowance for "extra sodium" the great majority of extracellular water figures in normal rats would fall below 150 ml. or 15 per cent of the whole tissue, and would range down to 11 per cent of the whole tissue. This range has some support also from the figures for interstitial fluid in muscle of dogs calculated by Peters (27, page 135) on the basis of Lepore's figures.

To carry along the effect of a 10 to 20 per cent change in extracellular water figures into the subsequent calculations would mean a  $2\frac{1}{2}$  to 5 per cent decrease in the concentration of potassium in the intracellular water. Since our figures from normal rats for potassium concentration in intracellular water seem to run somewhat high (154 to 178) as compared to those given for muscle and various other tissues in various animals (22) (muscle: one dog, 132; one monkey, 143; one rabbit, 122; other tissues: 109 to 155) it might be argued that the "extra sodium" allowance would bring them closer to other muscle figures. However, it might equally well be argued, as shown above, that it would bring the extracellular water figures out of line with those reported figures that are in the neighborhood of 17 to 20 per cent (Peters (27, pages 130, 134, 141)). Besides, our figures for concentration of sodium plus potassium in total water also average higher than other reported figures. Light, Smith, Smith and Anderson (28) give figures ranging from 125.4 to 176.6 on whole rat bodies, and Harrison, Darrow, and Yannet (22) show results for muscle of one dog, 144; one monkey, 149; one rabbit, 127; while for other tissues in these animals they give sodium plus potassium concentration ranging from 139 to 175. The range of 153 to 171 covers all of our sodium plus potassium concentrations per liter of total muscle water in normal rats. Our average in Table III is no higher than the one figure calculated from the Katz analysis of human muscle as shown by Peters (27, page 129) though the relative amounts of sodium and potassium are quite different from these figures for human muscle, and as Peters points out the Katz figures seem to run higher than other reported muscle figures. At any rate, for a comparative study as between normal and edematous rats, of the osmotic concentrations of potassium or sodium in their

respective compartments of water, the "extra sodium" can be disregarded.

### *Relation to edema*

In general, the relations of sodium and potassium to muscle water in our rats with a nutritional edema and hypoproteinemia are such as to indicate that the stored sodium and water can be entirely accounted for by an accumulation of ultrafiltrate of the plasma. The extracellular fluid per kilogram of tissue may reach an amount as great as three times the normal with no significant change in the osmolal concentrations in the two compartments of muscle water. This is the broad conclusion resulting from comparison of the values for sodium plus potassium per liter of total muscle water and for concentration of potassium per liter of cell water in the normal animals with the same values in the edematous animals. A more detailed analysis of these figures can be had from Figure 2, and from the following discussion of the tables. For the first series of edematous animals in Table II the average values are essentially the same as the complete average for the first series of normal animals in Table I, though there is more variation in these values among the edematous group. For values of concentration of potassium in cell water there are three higher than the highest normal and one ( $K_3$ ) so much lower than the lowest that there seems a possibility of technical error as mentioned above. For the second series of edematous animals, Table IV, these average values are 5 and 8 per cent higher than the same averages for the second series of normals, Table III. As before, there is also more variation than among the normal group but this second normal group itself shows much more variation than the earlier normals, and the average figure for sodium plus potassium concentration is 5 per cent lower, the potassium concentration 6 per cent lower than the corresponding figures in the first normal series. Were this last edematous series to be compared with the earlier normals then, it would hardly show any significant difference in average values. It is perhaps more reasonable to make the comparison with the group of normals which was dieted coincidentally and in which the procedure for analyses was the same. From this comparison it might be concluded that there is a tendency for slightly higher osmotic



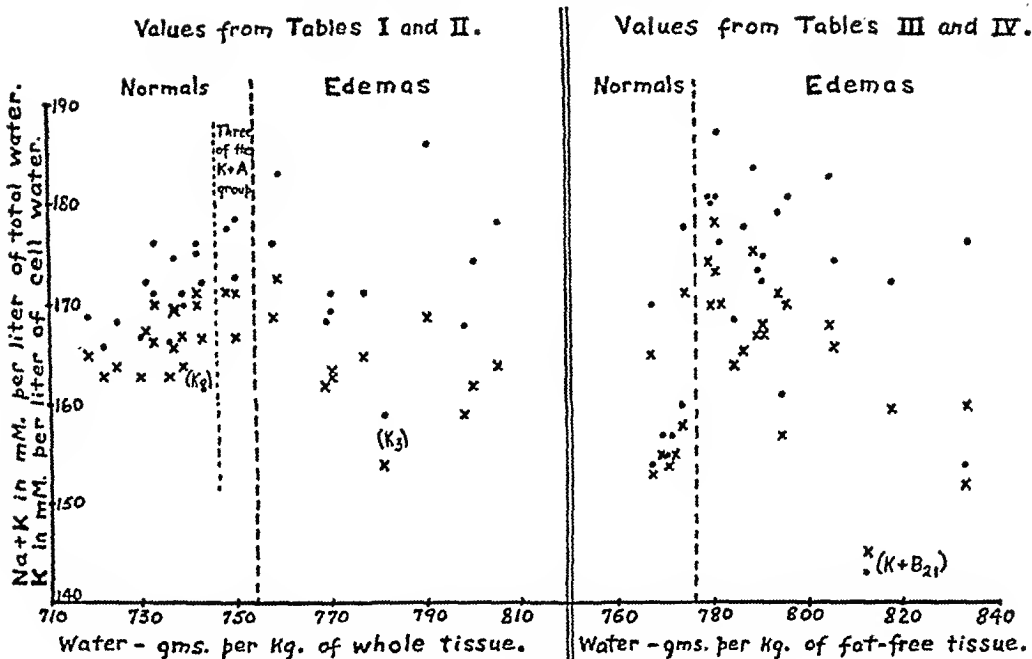


FIG. 2. THE CONCENTRATION OF MUSCLE BASES IN RELATION TO THE WATER CONTENT OF THE MUSCLE.

The crosses represent values for the concentration of Na + K in total water, and the dots, values for the concentration of K in calculated cell water.

concentrations in the total muscle water of edematous rats than in the muscle water of normal rats. This could be a reflection of a slightly higher than normal sodium concentration in the plasma of edematous rats, which might show its effect in the tissues as an increased sodium concentration in interstitial fluid, and, in consequence, by a shift of water out of the cells to satisfy osmotic equilibrium, an increased potassium concentration in the cell water. Darrow and Yannet (29, 30) have shown definitely that the shift of water between erythrocytes and plasma is such as to reestablish osmotic equilibrium when a change from the normal has occurred in the osmolal concentration of the plasma. As these authors and Peters (27, pages 144, 145) argue, the same is to be expected between other cells and the fluid surrounding them. Without serum sodium figures to establish a relationship between the muscle bases and the chief base of the serum, we cannot go beyond conjecture on this point. At any rate, the increased base concentration in these edematous rats is not out of physiologic range, and must theoretically be shared by both the intracellular and extracellular water. This would indicate that the

figure used for sodium concentration in the interstitial fluid of normal animals should have been slightly lower than that of the edema fluids. There seems to be no correlation of this tendency to higher base concentration with the degree of edema.

#### CONCLUSIONS

Lack of vitamin G is not significant for the occurrence of edema in young rats on a protein-poor carrot diet. The addition to the diet of as small an amount as 0.05 gram daily of a biologically good protein causes some delay in the edema formation.

The ratio of albumin to globulin in normal rat serum is equal to 1, as contrasted to approximately 2 in normal human serum. The loss of serum protein in rats with a nutritional edema is more at the expense of serum albumin than serum globulin, as in the human edema, so that the ratio of serum albumin to globulin becomes less than 1.

The calculated values for amounts of extracellular water in muscle with declining serum protein values indicate that the retention of fluid in the muscle begins with the first loss of serum



protein and progresses with increasing hypoproteinemia. This finding argues against the concept of a "critical level" of plasma proteins for the formation of edema fluid. The progressively greater accumulation of edema fluid in the muscle when the plasma protein drops below 4.5 per cent correlates with the gross findings in clinical edemas.

In general, the values of muscle sodium and potassium, and of muscle water in rats with a nutritional edema are such as to be accounted for by normal concentrations of sodium in extracellular water and potassium in intracellular water. An appreciable number of edematous animals did show a 5 to 8 per cent increased concentration for these two bases combined, but this is not out of physiologic range of osmolar changes. The theory that the accumulation of fluid in the tissues in conditions of hypoproteinemia is simply a result of ultrafiltration through the capillaries caused by the reduced plasma osmotic pressure is compatible with these determinations of the predominant bases in edematous rat muscles.

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# THE COMPOSITION OF HUMAN GALLBLADDER BILE AND ITS RELATIONSHIP TO CHOLELITHIASIS

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Chemical investigations of the activity of the gallbladder have aided notably in clarifying the etiology of gallstones. Detailed reviews have been published by Lichtwitz (1) and Ivy (45). Particularly important is the striking alteration of gallbladder function caused by infection of or injury to the mucosa demonstrated in the dog by the experiments of Drury, Rous and McMaster; Ravdin, Johnston and Riegel; and of Andrews and associates. It has been shown by these workers that changes in the composition of bile exposed to the action of pathologically altered gallbladders tended to diminish the effectiveness of the bile as a solvent for cholesterol. Thus, considerable experimental evidence has been presented to connect the development of gallstones with infection of or injury to the gallbladder mucosa. The most convincing support has been derived from experiments done upon dogs, although data have been published from the laboratories of Ravdin and of Andrews showing that similar processes appear to operate in the human gallbladder. Species differences, however, necessitate caution in drawing close analogy between observations made in the presence of experimental gallbladder injury of dogs and in human cholecystitis. As has been pointed out, human bile differs in composition from dog bile, and also, the response to disease and injury of the biliary tract in man is not quite the same as that of the dog under experimental conditions. Human bile is rich in cholesterol as compared with dog bile. It differs in type and amount of bile acid. Cholecystitis in the dog causes the gallbladder contents to become more alkaline, but in man the opposite effect is generally observed. Therefore, more complete investigation of additional human material is desirable to establish clearly the consequences of cholecystitis and its rôle in cholelithiasis, as well as to correlate more closely such observations with the studies on animals. The

results of a chemical study of human gallbladder bile are reported in this paper. The explanation of gallstone formation provided by the work of Ravdin, Johnston, Riegel and associates and of Andrews, Schoenheimer and Hrdina is supported by our data.

## PROCEDURE

Bile was aspirated directly from the gallbladder into a syringe by the surgeon in the course of operation. Precautions were taken to avoid exposure to air or loss of carbon dioxide. Preceding the operation, the usual saline enemas and morphine were given. Some of the patients received glucose intravenously in addition. Generally, the patients had fasted 12 to 15 hours before operation, occasionally somewhat longer.

Determinations of pH were completed within 1 to 2 hours after collection of the specimen. Other tests also were started with a minimum of delay. Occasional specimens could not be analysed promptly; pH determinations were then omitted. Analytical findings were not influenced appreciably by delays of several hours. The specimens were centrifuged and the precipitate, if any, examined for the presence of crystals or other formed elements. Measured amounts of centrifuged bile were added to warm absolute ethyl alcohol, heated to boiling, centrifuged, decanted, and the residue extracted twice with alcohol. The alcohol solution and washings were combined and diluted to volume in a volumetric flask. Aliquots were used for the determination of cholic acid, phosphorus and cholesterol. The absence of cholesterol esters from bile and the solvent action of the bile acids and phospholipid permit the use of alcohol alone as an extractive solvent for cholesterol. The latter was estimated by a modification of the Autenrieth and Funk method (2). Bile saponified two hours with 25 per cent potassium hydroxide was extracted with ether in a continuous extractor of the type described by Quick (3). Cholic acid was determined by the modification of the Gregory-Paseoc procedure developed by Reinhold and Wilson (4), using the precautions recommended for analysis of human bile. Turbidity resulting from the presence of desoxycholic acid was removed by addition of alcohol after color development as described in the method. Phospholipid values represent total phosphorus of the alcohol soluble components of bile.

In the presence of bile acid salts alcohol appears to dissolve completely bile phospholipid, although Mathews (5) states that alcohol precipitates cephalin ordinarily. Analytical methods that are not specifically mentioned are those listed by Reinhold and Wilson (6). Bile was ashed preliminary to determination of cations. To avoid possibility of error due to the high concentration of solids, water was added to the sample taken for the chloride determination, as suggested by Sunderman and Williams (7). Analytical results have been calculated on the basis of the concentration per liter of bile.

Attempts to study changes in the bilirubin content of bile of diseased gallbladders were hampered by the difficulties of determining this substance quantitatively. When possible, direct comparison of the diluted bile with solutions of potassium bichromate was made. The latter were standardized with the aid of solutions of pure bilirubin (0.025 per cent potassium bichromate was equivalent to 0.38 mgm. per cent bilirubin). Obviously, the method was not applicable when oxidation of the bile pigment had occurred.

The specimens analysed were derived principally from pathological gallbladders showing evidence of inflammatory disease. In addition, specimens from gallbladders showing no distinct pathological changes, as well as specimens from gallbladders presenting cholesterosis or neoplastic disease, have been examined.

To demonstrate the cumulative nature of the pathological changes in cholecystitis, data are arranged in Tables I to III according to the absence or presence and the severity of cholecystitis. Table I includes data for a group of control specimens. In Tables II and III are shown figures for specimens taken from gallbladders exhibiting moderate or marked evidence of disease, respectively. When possible, classification has been made upon histological evidence. When the gallbladder was not removed or examined, the conclusions of the surgeon concerning the condition of the gallbladder were accepted. Histologically, thickening of the walls, extent of fibrosis, number and quality of villi, and presence and degree of inflammatory reaction and cellular infiltration have been given the most weight. Tabulated according to these criteria, fair homogeneity is observed in each group, while significant differences between the groups likewise become apparent. An alternative grouping, employed in an earlier study (Reinhold and Ferguson (8)) and considered in connection with the classification of the present material, depended on the

presence of calculi in the gallbladder or of both calculi and obstruction. Actually, the difference in distribution of specimens according to the two plans was not great, since only 5 of 35 gallbladders showing evidence of inflammatory changes were without calculi, and only 2 failed to show crystals of cholesterol.

The calculi encountered were almost all of the cholesterol calcium pigment type or of the cholesterol type. It was not always possible for the authors to examine the stones removed. The analytical findings of Ray (9) and of Riegel, Ravdin, Johnston and Morrison (47) indicate that there is less difference in composition between various types of calculi than had been supposed.

#### *Composition of bile from gallbladders normal in appearance*

Table I includes observations made upon bile from gallbladders without perceptible pathological lesions or with slight changes. Laparotomy was undertaken either for exploration or because of disease not involving the biliary tract, although gallbladder disease was suspected in several patients due to the association of certain characteristic symptoms with failure to visualize the gallbladder by the cholecystographic technic of Kirklin (51). The group is heterogeneous, and there exists a distinct possibility that abnormalities of bile secretion and of gallbladder function influenced the composition of some specimens. Bile from normal living individuals, however, is rarely available. The specimens have been arranged according to cholate concentrations in descending order, since it is reasonably certain that ability to concentrate bile salts is an especially significant indicator of functional capacity. Thus, specimens in the upper half of the table are most nearly normal. It is evident that figures for some specimens in the lower portion overlap data for specimens from patients suffering from cholecystic diseases. Graham and Mackey (50) have pointed out difficulties of confirming at times by histological methods the presence of gallbladder abnormalities. It is possible or even probable that gallbladder disease existed unrecognized in this group. Adhesions involving the gallbladder existed in 6 patients. Crystals, either of cholest-

TABLE I  
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Case number	Operative findings. Remarks	Biopsy †	Color	Stones or crystals †	Solids per cent	pH	HCO <sub>3</sub> m. eq. per liter of bile	Chloride m. eq. per liter of bile	Cholate m. eq. per liter of bile	Sodium m. eq. per liter of bile	Potassium m. eq. per liter of bile	Calcium m. eq. per liter of bile	Magnesium m. eq. per liter of bile	Undetermined anion m. eq. per liter of bile	Bilirubin m. l.	Lipoid phosphorus m. l.	Cholesterol mgm. per cent	Cholate Cholesterol
26	Adhesions to gallbladder	a	Brown	0	18.4		10.0	27.8	206	307	16.0	34.3	25.2	139	14.9			16.0
39	Adhesions. X-ray dye test normal on second trial	0	Brown	P	16.2		18.5	50.0	174	259	11.7	23.5	19.5	72	5.2		450	6.2
45	Cystic lymphangioma of peritoneum	0	Brown	Cr, P	18.6		6.7	9.9	162	280	14.5	29.1	20.9	174	6.0		1010	11.1
34	Normal appearing gallbladder	0	Dark brown	0	16.0	6.95	13.1	44.0	161	254	10.5	17.1	17.6	82	1.1		540	
61	Normal appearing gallbladder	0	Brown	Cr	15.5		16.8	15.0	136						32.9	66.5	370	10.5
58	Typhoid carrier. X-ray dye test: no visualization	a	Brown	Cr	15.5		15.1	21.6	121	231	15.0		18.2	129*	6.1	36.4	370	3.8
43	Cystic duct kink	a	Yellow	P	15.0		27.3	33.3	96	253	7.7	19.3		142			320	10.3
16	Adhesions. X-ray dye test: no visual- ization	b	Brown	0	14.6	7.21	31.2	39.6	89	257	16.5	26.7	35.4		8.5		466	20.5
33	Diodenal ulcer. Gallbladder adhesions	0	Brown	Cr	11.4	6.90	9.9	65.8	85	223	9.2	13.2	8.3	85	1.9		160	21.3
30	Adhesions to gallbladder	0	Brown	0	12.1	7.24	16.7	54.0	85	240	9.1	16.3	9.4	118	5.1		140	
21	Adhesions. Cholesterol deposits	b	Brown	0	12.6		38.0	50.1	68	224	13.9	18.8	13.8	114			260	10.7
23	Normal appearing gallbladder	0	Brown	0	13.5	7.19	20.5	50.8	67	238	6.6	29.7		153			260	10.5
9	X-ray dye test: no visualization	a	Brown	0			27.9	58.5	48									

\* Starred values are estimated by supplementing determined values with average value found within group.

† a, No pathological changes.

b, Slight pathological changes.

c, Moderate inflammatory change.

d, Marked inflammatory change.

0, No specimen.

S, Cholesterol, calcium, pigment calculus.

Cs, Cholesterol calculus.

Cr, Cholesterol crystals.

P, Pigment crystals.

-S, Calculus present, type not known.

TABLE II  
*Moderate cholecystitis* †

Case number	Operative findings. Remarks	Biopsy	Color	Stones or crystals	Solids	pH	HCO <sub>3</sub>	Chloride	Cholate	Sodium	Potassium	Calcium	Magnesium	Undetermined anion	Bilirubin	Lipid phosphorus	Cholesterol	Cholate
19	Slight thickening of gallbladder.	c		S, Cr			18.2	76.8	17								330	2.1
24	Chronic cholecystitis				7.50	7.14	9.3	75.0	32	179	7.7			101*				
31	Slight thickening of gallbladder.	b		-S														
64	Chronic cholecystitis	b, c	Yellow	S, Cr	8.4	7.20	24.7	62.0	46	200	7.0	18.4	13.5	96	4.9		430	4.0
53	Slight thickening of gallbladder. Villi well formed. Adhesions	b, c	Dark brown	Cr			27.8	73.2	30.4									
3	Intermittent obstruction	0		S, Cr	12.1		14.6	62.8	51	174	14							
13	Cystic duct obstruction	0	Brown	S	14.2	6.97	14.3	59.3		203	8.3	31.4	19.6				390	3.0
15	Cystic duct obstruction	c	Dark brown	Cs, Cr	19.6		25.3	56.0	35	214	6.3	53.3	20.6	175		26.5	460	4.3
22	Cystic duct obstruction. Chronic cholecystitis	c	Yellow	S	8.5	7.14	31.4	85.0	55	205	7.5	16.4	22.5	80	2.3	5	173	
47	Recurrent cystic duct obstruction. Subacute inflammatory changes	b, c	Gold brown	S, Cr	5.8		28.0	91.5	19	168	7.2	8.0	4.7	50			320	2.5
11	Cholecystitis. Hepatic cyst		Dark brown	S, Cr	7.7		21.8	88.6	30	148	8.3	14.9	6.9	23	20.2		200	6.0
11a	Fluid from hepatic cyst	0	Dark brown	S, Cr	12.6	6.98	26.3	58.1	63	246	13.5	24.2	11.4	148	4.4			
17	Moderate chronic cholecystitis	c	Brown	S, Cr	7.98	5.86	1.6	116.1	0									
27	Moderate chronic cholecystitis	c	Brown	S, Cr	7.1		27.8	81.0	34	179	5.8	8.5	7.2	58	4.1		280	4.7
40	Moderate chronic cholecystitis	c	Brown	S, Cr			9.3	96.0	41									
56	Typhoid carrier. Chronic chole- cystitis. Thickened mucosa	c, d	Brown	-S, Cr	7.7		24.5	72.0	27	178	9.1	11.7	7.4	82	7.8	20	140	10.0
46	Obstruction. Acute cholecystitis	c	Brown	S, Cr	6.5		15.0	90.7	25	172	7.0			58*			240	4.0
29	Obstruction. Chronic cholecystitis	c	Red brown	S, Cr	2.0		49.0	103.7		156	5.4	4.2	1.5	45	0.3	21.5	40	
55	Obstruction. Thick gallbladder mu- cosa. Edema. Villi preserved	c	Turbid green brown	S, Cr	5.9		11.0	107.9	31	172	5.7	12.4	4.4					
					18.5		18.6	43.2	99	162	5.8	13.8	11.7	33		74	850	4.5

† For legend, see Table I.

TABLE III  
Severe cholecystitis †

Case number	Operative findings. Remarks	Biopsy	Color	Stones or crystals	Solids	pH	HCO <sub>3</sub>	Chloride	Cholate	Sodium	Potassium	Calcium	Magnesium	Undetermined anion	Bilirubin	Lipid phosphorus	Cholesterol	Cholate
					per cent		m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.M.	m.M.	mgm. per cent	
25	Chronic cholecystitis	d	Brown	Cr	12.5		32.8	56.8	23	225	8.8	22.8	9.4	86			390	2.5
48	Profuse ulceration	d		Cr	11.7	6.98			25.1	201	3.0	22.4	12.9	95	1.5		600	2.8
10	Edema, thickened mucosa, cell infiltration	d	Brown	S, P, Cr			19.1	66.6	94									
41	Wall entirely fibrous	d		S			23.9	79.3	41							50.7		
36	Edema, ulceration. Acute and chronic changes	d	Pale green	0	5.7	6.85	7.5	121.2	0.8	142	5.1	7.4	2.0	27	0			
4	Empyema, obstruction, cystic	0	Dark brown	S, pus	9.0	6.67	13.5	78.2	48	171	6.4	12.0	5.3	54				
12	Obstruction, empyema	0	Green brown	Cr, Cs	6.3		3.6	65.2	2.6	136	7.2	11.7	4.0	88				
18	Obstruction, edema, acute and chronic inflammatory changes	p		S, Cr	10.3		42.5	75.8	37	186		14.6	8.5	54*	1.5		220	7.0
37	Obstruction, edema, acute and chronic inflammatory changes	d	Gold brown	S, Cr	4.3		38.6	106.0	10	154	7.8	6.7	4.4	18	0		120	3.5
44	Obstruction. Hydrops	d	Colorless	S	2.8		12.6	114.2	0.1		4.5	5.5	2.5				110	.02
52	Obstruction. Edema	p	Green brown	S, Cr	5.9		1.5	135	3.9	136						2.1	120	1.3
57	Obstruction. Edema. Newly formed soft calculus. Recent pregnancy	d	Turbid green	S, Cr, P	6.1		13.0	112.5	13	156	5.0	7.8	4.5	35		11.3	160	3.1
35	Obstruction. Thick fibrous wall	0	Dark green	-S	3.4	7.05	11.1	101.9	18	144	6.2	7.9	3.0	30		26.5	100	7.3
62	Obstruction. Thick wall	0	Brown	S, Cr				85.6									240	

† For legend, see Table I.



terol or bile pigment, were found in 5 specimens, although always in small numbers. Neither crystals, adhesions, nor failure of visualization by x-ray, could be correlated with distinctive changes in composition of the bile specimens. However, Johnston et al. (10), and Riegel and co-workers (47), by repeated testing, frequently with larger amounts of dye, have succeeded in demonstrating chemical differences between specimens secured from gallbladders exhibiting normal response to cholecystography from those without calculi that failed to do so. The specimens ranking near the top in Table I varied only slightly in regard to calcium, chloride and bile salt concentrations from two samples of normal human gallbladder bile analysed for these constituents by Johnston, Ravdin, Riegel and Allison (10). Concentrations of bile acids in those specimens we consider most nearly normal (the first 7 in Table I) range far above all except two values reported by Andrews (11) for "normal" gallbladder bile. It is doubtful whether any of the specimens labeled normal by this author should actually be so considered.

All specimens listed in Table I contained high concentrations of solid material. This is largely bile acid and phospholipid, if the two figures for the latter may be considered representative. Determined bile acid, glycocholate and taurocholate, often accounted for more than half the total solids. The high concentrations of these substances are to be contrasted with the far lower concentrations found when the gallbladder mucosa is perceptibly injured. Owing to the fact that the modified Pettenkofer reaction employed for the determination of bile acids is specific for the cholic acids (Reinhold and Wilson (4)), other bile acids that occur in human bile are included in the undetermined anion.<sup>1</sup> Desoxycholic acid appears to be most important quantitatively, according to Wieland and Reverey (12) and Doubilet (13). Chenodesoxycholic acid (12) and lithocholic acid (14) have been isolated from human bile and from gallstones, although data are not available relative to their concentration. It is to

<sup>1</sup> Doubilet (13) has justly pointed out that data for human bile based upon this method alone are incomplete. In the present study this objection is avoided because changes in the undetermined anion offer means for detecting gross changes in other bile acids.

be expected that differences between normal and pathological specimens resembling those seen for the cholic acids exist also for the undetermined bile acids. High figures for undetermined anion in the normal and moderately pathological specimens do indeed support this conclusion although the contrast is not as clearly defined. That there is a change in the character of bile acids secreted under certain abnormal conditions is suggested by the work of Greene, Walters and Fredrickson (15), of Ravdin, Johnston, Riegel and Wright (16), and of Breusch and Johnston (17). Doubilet (13) recently has demonstrated such a qualitative alteration. Doubtless the high undetermined anion concentration, frequently observed in our series in conjunction with relatively low cholate, is the result of a similar change in the type of bile acid secreted by the liver.

Chloride concentrations in specimens from gallbladders without demonstrable pathological changes were considerably less than the concentrations of organic anions. The relatively low chloride figures may be contrasted with the high values observed in pathological material described in the next section. Similar differences have been observed by Johnston et al. (10). The concentration of chloride varies inversely with that of bile acid and compensates changes in the latter. Bicarbonate undergoes somewhat similar alterations, and it also may replace or be replaced by bile acid. Bicarbonate rarely exceeds chloride in concentration and ordinarily it is considerably less. While high bicarbonate has occasionally been found associated with diminished chloride concentrations, generally both are increased or lowered together.

The concentration of cation in bile likewise is governed to a considerable extent by the level of bile acid; consequently, sodium and other cations as well are relatively high in the specimens of this group. Sodium is by far the most important of the cations so far as concentration is concerned, with calcium, magnesium and potassium ranking in the order mentioned when considered in terms of milli-equivalents per liter. Magnesium concentrations in bile frequently outweigh those of calcium in contrast to serum where magnesium approximates only one-half of the calcium concentration. Furthermore, the divalent cations

of bile constitute a considerably larger fraction of the total base than in serum. Concentrations of all cations of bile normally exceed by considerable amounts the concentrations in serum, while the total cation concentration is consistently above 250 m.eq. per liter of water as compared with the average concentration in serum of 167 m.eq. Despite this discrepancy, it has been demonstrated repeatedly that bile and serum are iso-osmotic (see Brand (48), Strauss (49), Ravdin, Johnston, Riegel and Wright (16), and Gilman and Cowgill (18)). The anomaly is a manifestation of the abnormal behavior of ions of low molecular weight in the presence of large ions. Hammarsten (19) has observed that in the presence of bile acids, as well as of other compounds of high molecular weight, osmotic activities of smaller ions are diminished.

The reaction of bile from gallbladders showing no evidence of disease was found in an earlier investigation by two of the writers (Reinhold and Ferguson (8)) to vary between pH 7.10 and 7.30. Although it was suggested that greater variation was to be expected, in the present series 3 of 5 similar specimens fell within these limits. The 2 remaining specimens were somewhat more acid in reaction, the pH being 6.90 and 6.95. The range of values for specimens of this class must be broadened to include the latter. Low pH was associated with low concentrations of bicarbonate.

Comparison of pH values reported for dog and human bile shows that gallbladder bile of dogs has been found to be somewhat more acid in reaction (Okada (20); Drury, McMaster and Rous (21); unpublished observations of the writers) than human gallbladder bile (Drury, McMaster and Rous (21); Reinhold and Ferguson (8); Andrews (11)). Undoubtedly, differences in the nature and concentration of the species-predominant bile acids are responsible. Human bile contains principally the relatively weak glycine-conjugated cholic and desoxycholic acids. Bile acid of the dog, on the other hand, consists primarily of the strong taurine-conjugated cholic acid. It has been shown that the pH of dog bile is governed by the concentration of taurocholic acid (Reinhold and Wilson (6)). In human bile, because of the relatively weak character of the bile acids, such a relationship is not clearly dem-

onstrated. Interpretation of the rôle of bile acid in the regulation of the reaction of human bile is complicated by the appreciable concentration of such acids included in the undetermined anion.

Carbon dioxide tensions of gallbladder bile frequently are very high. Calculation was made from the data for pH and carbon dioxide concentration by the Henderson-Hasselbalch equation according to Peters and Van Slyke (22). Values of the same magnitude have been established for urine by Sendroy, Seelig and Van Slyke (23), while contents of intestinal loops also may have high carbon dioxide tensions according to deBeer, Johnston and Wilson (24). It would appear that carbon dioxide tensions of secretions may differ appreciably from those of blood.

In normal bile, total cation concentrations consistently exceed the concentrations of total determined anion. The undetermined anion, as already indicated, is made up chiefly of bile acids other than cholic acid, although certain phospholipids may bind a portion of the base represented in this fraction. Inorganic phosphorus, if present, exists in amounts insignificant by comparison with other anions. None was found in dog bile (Reinhold and Wilson (6)). The presence of appreciable concentrations of inorganic phosphorus in bile has been reported; however, this may have originated by hydrolysis of phospholipid. Riegel et al. (47) found little phosphate in human biliary calculi.

Aronsohn and Andrews (25) have attempted to explain the acidification of bile in the gallbladder as a consequence of increased concentration of phosphorus and protein. Their conclusion, based on an increase in total phosphorus in gallbladder bile as compared with fistula bile, is not valid since the total phosphorus consists almost entirely of phospholipid. The latter, according to Hammarsten (26), is chiefly lecithin. As lecithin does not exert appreciably acidic properties at the pH of gallbladder bile (Fischgold and Chain (27); Chain and Kemp (28); Jukes (29)), it is not permissible to assign to the phosphorus of bile the acidifying action observed. Likewise, it appears improbable that bile protein would possess the powerful acidifying action implied by Aronsohn and Andrews. As already noted, there

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is considerable evidence showing that bile acid concentration is the chief factor regulating the pH of bile, and that acidification of gallbladder bile is primarily a result of the large increase in bile acid concentration as hepatic bile is concentrated in the normal gallbladder.

Cholesterol in these specimens from gallbladders without pathological changes varied in concentration between 140 and 1010 mgm. per cent. The highest value cited was also the highest in the entire series. The patient suffered from cystic lymphangioma of the peritoneum. Despite the elevated concentration of bile acids, cholesterol crystals were detected in this specimen. Also, in two other specimens where cholesterol concentrations were not elevated, a few cholesterol crystals were seen although bile acid concentrations were high. It must be conceded, however, that crystals could not be found in the abundance that is ordinarily characteristic of bile from pathological gallbladders. The importance of the ratio between bile acid and cholesterol as a factor governing the solubility of cholesterol has been stressed by Newman (30), Andrews, Schoenheimer and Hrdina (31), and Johnston et al. (10). Such a relationship between cholate and cholesterol may be seen in the present series. It is noteworthy that only two specimens of this normal group had a cholate: cholesterol ratio of less than 10. The high undetermined anion of these non-conforming specimens suggests that the ratio of total bile acid to cholesterol would be considerably higher.<sup>2</sup>

<sup>2</sup> Other substances in bile beside cholesterol may react to give color with the Liebermann-Burchard reagents. Thus Wright (32) found the colorimetrically determined values for cholesterol in dog bile to be higher by 20 per cent than the results of determination by digitonin precipitation. Wieland and Reverey (12) report that anthropolidesoxycholic acid gives a weak Liebermann-Burchard reaction. While the term cholesterol has been used in the text and tables, it should be regarded as an expression of total non-saponifiable material giving the Liebermann-Burchard reaction. For the purposes of the present paper, there is reason to believe that the "cholesterol" values approximate cholesterol concentrations without gross inaccuracy. Comparisons with gravimetric digitonin determinations showed that colorimetric estimations did not vary more than 10 per cent from the digitonin values.

### *The composition of bile from diseased gallbladders*

Distinct changes from the normal composition of bile accompanied even slight pathological alterations of the gallbladder mucosa. The effects of cholecystitis of mild or moderate degree are shown in Table II. Gallstones were present in 16 of the 18 gallbladders from which these specimens were removed, while all of the bile specimens contained cholesterol crystals. Obstruction of the cystic duct was found at operation in 9 of the patients represented in this group.

Severe cholecystitis, with widespread changes in the mucosa, caused alterations in the composition of the contents of the gallbladders similar to those observed in moderate gallbladder disease, though far more pronounced. Data for 15 specimens of this character are presented in Table III. Here, likewise, the incidence of calculi was high. However, two specimens contained no crystals, nor were calculi found in the gallbladders. Partial or complete occlusion of the bile ducts caused by calculi existed in 10 of the 14 patients. Changes from the normal were most marked in the presence of obstruction. The group was far from homogeneous in respect to appearance of the specimens, which varied from essentially normal pigmentation to practically pigment-free "white bile."

The lowered concentration of total solids to be seen even in the presence of moderate injury to the gallbladder (Table II) reflects a marked impairment of the ability to retain and concentrate material reaching the gallbladder in the hepatic bile. Severe damage to the gallbladder mucosa depressed the solids content of the bile to a greater extent (Table III). Particularly striking changes were associated with obstruction of the cystic and common bile ducts. Estimation of the protein contained in certain of these specimens showed increased concentrations in both groups as compared with normal specimens. Thus the loss of ability to concentrate bile as measured by determinations of total solids is obscured to some extent by elevated protein, and was greater than the figures for total solids would indicate.

Striking reductions in concentration of bile acids in bile in the presence of gallbladder disease have been reported (10, 11, 30, 31, 47). Our

analyses likewise showed that cholic acid concentrations were lowered consistently in specimens comprising this group. The loss of bile acid explains the major portion of the decrease in solids. Whereas cholate in normal specimens exceeds 150 m.eq. per liter, the average concentration in the presence of gallbladder disease was reduced to 34 m.eq. per liter (Table II). Under similar conditions, undetermined anion concentrations were generally below the normal level although not as much so as cholate. It seems probable that undetermined bile acids behaved not unlike the cholic acids. Even lower concentrations of bile acids were observed in bile from badly diseased gallbladders (Table III), and in a few specimens, particularly in the presence of obstruction and empyema, only traces of cholate

the gallbladder, equivalent quantities of inorganic anions, largely chloride, replace the organic electrolyte so lost. Excessive concentrations of chloride are observed in the presence of marked edema of the gallbladder mucosa when the chloride concentration of the contents often exceeds that of serum. Riegel, Ravdin, Johnston and Morrison (33) have reported high chloride concentration in hydrops fluid. The importance of increased chloride as an indicator of gallbladder dysfunction has been stressed by these workers (10).

Bicarbonate, like chloride, may replace bile acid that diffuses from the injured gallbladder. In certain of the specimens represented in Tables II, III, IV and V, bicarbonate is increased in comparison with the normal while a few genuinely high values are seen. However, in the most

TABLE IV  
Cholesterosis †

Case number	Operative findings. Remarks	Biopsy	Color	Stones or crystals	Solids	HCO <sub>3</sub>	Chloride	Cholate	Sodium	Potassium	Calcium	Magnesium	Undetermined anion	Bilirubin	Lipoid phosphorus	Cholesterol	Cholate/Cholesterol
					per cent	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	mM.	mM.	mgm. per cent	
49	Cholesterosis	b, c		Cs	8.1	63.1	32.1	23									3.8
28	Cholesterosis	c		Cr		22.3	82	36	177	4.0	11.8	9.8	61			240	
54	Cholesterosis. Gangrenous pancreatitis	c	Yellow	S, Cr	12.2	15.1	38.6	76	137						9.3	192	16.1

† For legend, see Table I.

could be detected while other bile acids likewise appeared to have vanished. Heightened permeability of the gallbladder mucosa which ceases to act as a barrier to bile salts and perhaps to other substances of high molecular weight undoubtedly is foremost among the factors leading to such changes. There is little doubt that the lowered concentration of bile acid diminishes the solvent action of the bile for lipids, and leads in turn to crystallization of cholesterol.

High chloride concentrations were associated consistently with the low bile acid concentrations typical of cholecystitis. Normally, as the concentration of bile acid increases, chloride diffuses from the gallbladder, thus maintaining osmotic equilibrium. However, in the presence of moderate cholecystitis the relatively high concentration of chloride present in hepatic bile not only remains undiminished, but, as bile acid diffuses from

severely damaged gallbladders bicarbonate is more often decreased. It appears that the abundant secretion of bicarbonate in the dog described by Ravdin, Johnston, Austin and Riegel (34) as a consequence of injury to the mucosa is not the prevailing response to chronic cholecystitis in the human. Perhaps it represents an early phase in the response to injury, although the occurrence of calculi composed of calcium carbonate implies a persisting increase in bile pH.

Although but little change in pH of bile accompanies moderate gallbladder disease, a shift toward more acid reactions commonly is associated with marked injury to the walls of this organ. In this respect, data obtained in the present study confirm the earlier observations of the writers.

Decreased pH and bicarbonate under these circumstances provide an interesting contrast to the

TABLE V  
Carcinoma involving biliary tract †

Case number	Operative findings. Remarks	Biopsy	Color	Stones or crystals	Solids	pH	HCO <sub>3</sub>	Chloride	Cholate	Sodium	Potassium	Calcium	Magnesium	Undetermined anion	Bilirubin	Lipid phosphorus	Cholesterol	Cholate
					per cent		m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	m.eq. per liter of bile	mM.	mM.	mgm. per cent	
8	Obstruction. Carcinoma of gallbladder	0	Gray green turbid	S	6.3	6.60	3.4	119.0	129	2.9	6.6	1.5					60	0.3
1	Obstruction. Carcinoma of ampulla	0		0	8.29		47.1	61.5	161								175	0.8
2	Obstruction. Carcinoma head of pan-	0		0	7.83		9.9	105.0	169									
32	Obstruction. Carcinoma head of pan- creas. Cholesterosis. Diverticulum	0	Pale yellow	Cr	1.65	7.24	21.2	110.9	0.4	2.5	3.4	1.8			9.7	1.1		
42	Obstruction. Carcinoma head of pan- creas	0	Yellow	S, Cr micro- liths	2.99	6.91	11.2	149.8	3.7	4.0	7.7	3.3			14.9	6.4	1.5	10
50	Obstruction. Carcinoma head of pan- creas	0	Colorless	C	0.92		4.0	139.2	0.4	2.8							4.8	22.6
50a	Contents of common duct gall- bladder enlarged, thickened.	0	Brown	Cr, P			14.0	128.0	0.5									
60	Obstruction 5 months																	

† For legend, see Table I.



changes observed in the gallbladder bile of dogs following the development of cholecystitis. Drury, McMaster and Rous (21), and Johnston et al. (10) have shown that injury to the mucosa of the gallbladder causes the pH and bicarbonate of dog's gallbladder bile to increase. Again, the differences in the chemical properties of the predominating bile acids in human and dog bile aid in explaining the inconsistency. Salts of the weakly acidic glycine-conjugated acids of human bile on undergoing hydrolysis would impart a slightly alkaline reaction to solutions. As already mentioned, the slightly alkaline reaction of normal human gallbladder bile is a consequence of this property. On the other hand, when glycocholic acid diffuses from the diseased gallbladder and is replaced principally by chloride, the reaction will tend to become less alkaline. In contrast, the strong taurine-conjugated bile acids of dog bile normally displace practically all of the bicarbonate (and chloride) as concentration proceeds in the gallbladder so that ordinarily the reaction is distinctly acid. Under these circumstances loss of bile acid from the gallbladder of the dog in the presence of cholecystitis, particularly if accompanied by secretion of bicarbonate (see Ravdin et al. (34)), will cause the marked increase in bile pH observed in this species. Impaired buffering capacity facilitates changes in reaction that occur in injured gallbladders.

The lowered pH of the contents of severely damaged gallbladders indicates that acidic constituents are present in significant concentrations. These are not cholic acids, free or conjugated, since low cholate is found in this class of specimens. Appreciable amounts of undetermined anion are observed. Its identity has not been established. That it is not phospholipid is shown by simultaneous occurrence of very low bicarbonate with the smallest phospholipid concentrations of the entire series.

Diffusion of bile acid out of the diseased gallbladder is only partially compensated by the rise in chloride (with or without a rise of bicarbonate). There is invariably a simultaneous and roughly proportional decrease in concentration of total base. While quantitatively smaller than the alteration of the chloride, it is nevertheless of sufficient size to bring about marked reduction of

the total cation concentration of the pathological specimens, as well as that of individual cations. Sodium, being the principal cation, accounts for the major portion of the change. However, calcium concentrations do not change to the same extent as a concentration of other cations.

The presence of considerable amounts of calcium as a constituent of many gallstones arouses interest in the concentration of this ion in bile of diseased gallbladders. Marked irregularity is shown in the response of calcium concentration of bile to gallbladder disease. Alterations that have been observed are most frequently in the direction of lower values. Occasional high concentrations were found in the contents of certain badly diseased gallbladders. Calcium concentration in normal bile varies directly as the concentration of bile acids, and no doubt a relationship exists analogous to that between serum calcium and serum protein. Thus high concentrations of bile acid would exert a protective action tending to keep calcium in solution by diminishing ionization. On the contrary, low bile acid levels would favor precipitation of calcium, thus contributing to a widespread occurrence of calcium in calculi. Inouye and Ryuichi (35) found that sodium taurocholate prevented precipitation of calcium although glycocholate was ineffective.

In disease, the changes in bile calcium are complex. Quite probably high calcium concentrations in the presence of severe cholecystitis are to be attributed to exudation or transudation. This explanation is supported by the simultaneous increase in the protein of the bile shown by unpublished observations of the writers. Riegel et al. (33) refer to the process by which calcium is increased as secretion. Phemister, Day and Hastings (36) have presented interesting examples of extreme accumulation of calcium in the gallbladder.

In cholecystitis, concentrations of bile magnesium are diminished. The calcium-magnesium ratio of the gallbladder contents ordinarily is lowered in comparison with that observed in normal specimens, approaching more nearly the ratio that characterizes blood serum or transudates. Potassium, like sodium, also approaches values commonly found in serum.

Contrary to expectations, cholesterol concn-



trations are lower in our specimens from pathological gallbladders, as judged by averages, than in the normal material. Differences become greater as the severity of injury to the mucosa increases, until the average cholesterol of Group IV (marked cholecystitis) is about half that of the normal. Between these extremes fall the averages of the intermediate groups. Individual variations within all groups are large. The low figures undoubtedly reflect a decreased capacity of the bile for holding cholesterol in solution.

These results are in agreement with those of Newman (30), Spanner and Bauman (37), Andrews et al. (31) and Riegel et al. (47) in showing that the underlying cause of the crystallization of cholesterol is not a primary excess of this substance, but rather a defect of the solvent. The obvious lowering in the ratio of cholic acid to cholesterol in pathological specimens, as compared with normals, provides a quantitative measure of this change. Our ratios are lower than those of certain other workers who employed the less specific determination of hydrolyzed amino-nitrogen for analysis of bile acids. In general, however, the results are similar. Despite the high cholesterol concentrations of many normal specimens, crystals of cholesterol were seldom encountered in such specimens. On the other hand, it appears that crystals and calculi rarely are absent from the contents of diseased gallbladders and usually are present in great abundance despite the lowered concentration of dissolved cholesterol.

Bilirubin solubility, like that of cholesterol, must depend upon the lipid-bile acid relationship since gallbladder bile usually lacks the alkalinity requisite to maintain bilirubin in aqueous solution. Furthermore, the activity of calcium undoubtedly increases as bile acid concentrations decrease. Thus loss of bile acid and lipid with the further likelihood of an increase in calcium concentration and activity may readily bring about precipitation of bilirubin. Possibly, because of the limitations of the method, it has not been possible to relate the occurrence of gallstones with the incidence of either high or low concentrations of bilirubin. The concentration of bilirubin in bile, as determined by the procedure described, varied widely both in the normal and in the several groups of pathological specimens. However, low concen-

trations were characteristic of the specimens from the most severely damaged gallbladders, as one might expect on the basis of physical factors. Not infrequently, crystals of bile pigment, the so-called calcium-bilirubin crystals, were present in specimens of all groups. Their presence appeared to be independent of concentration of bilirubin, calcium or hydrogen ions. The close correlation with the occurrence of cholecystitis that was found to exist in the case of cholesterol crystals was not observed.

The investigations of Fürth and Scholl (38) have again demonstrated the importance of phospholipid in the presence of bile acid as a solvent for cholesterol. Since bile is rich in lecithin and perhaps other phospholipids as well, these substances undoubtedly exert an important influence on the properties of bile as a solvent. An attempt has been made to determine the approximate concentration of phospholipid in the various specimens for the purpose of discovering whether cholecystitis led to any significant alterations in these substances. It appears that, like cholesterol, lipid phosphorus tends to remain in the damaged gallbladder while bile acid diffuses away. However, being comparatively unstable and perhaps more readily soluble than cholesterol, these substances would not persist as concretions as cholesterol does.

Protection of the gallbladder against the deleterious effects of bile salts is undoubtedly an important function of the lipids of bile. An injurious action of aqueous bile acid solutions on the gallbladder mucosa has been described by Riegel, Ravdin and Johnston (39), and Aronsohn and Andrews (25). Riegel and associates have also described a protective action of cholesterol against bile salts, while Ishii (40) also has found a similar protective action of phospholipid against bile salts.

*Cholesterosis.* No characteristic chemical composition of bile could be related to the presence of cholesterosis (strawberry gallbladder). While changes from the normal exist (Table IV), these were in general similar to those observed in cholecystitis of corresponding severity. The bile cholesterol was not elevated.

*Neoplasm.* The composition of the contents of the gallbladder in the presence of malignant

growths involving the biliary tract is shown in Table V. Obstruction of the cystic or common bile ducts existed as well in all of these patients. Despite the absence in most cases of conspicuous gross or microscopic changes in the gallbladder, the composition of its contents was profoundly altered. Such a finding suggests that obstruction may alter the composition as well as the secretion of bile. It is known that suppression of bile secretion follows obstruction of the common bile duct. Contrasting with the remainder of the group is Specimen 60, where malignancy and obstruction of possibly as long as 5 months' duration had no effect other than marked inspissation of the bile.

*Hepatic cyst.* An hepatic cyst was encountered in Patient 11. Fluid was aspirated from the cyst, and, after removal of leukocytes by centrifugation, was analysed by the methods used for bile. The specimen was colorless but distinctly opalescent. Cultures were negative. The specimen contained 7.89 per cent solids, 118.1 m.eq. per liter chloride, 1.6 m.eq. bicarbonate; the pH was 5.86. The resemblance of these figures to those found for the contents of severely damaged gallbladders is at once evident.

#### DISCUSSION

Diminished concentration of bile acid, as typified by cholic acid, proves to be an almost invariable accompaniment of injury to the gallbladder mucosa. This close association provides evidence of increased permeability of the wall of the gallbladder to bile acid, while loss by diffusion best explains the low bile acid concentrations. However, in the presence of injury to the hepatic parenchyma (Doubilet (13); Andrews, Hrdina and Dostal (41)), in pregnancy (Riegel, Ravdin, Morrison and Potter (42)) or obstruction of the bile ducts (Goff, Hrdina and Andrews (43); McMaster, Broun and Rous (44); Greene, Walters and Fredrickson (15)), alteration or suppression of bile acid secretion may be additional factors. Cholesterol and, presumably, phospholipid, fail to diffuse from the damaged gallbladder as does bile acid. The failure of phospholipid to form deposits like those of cholesterol may be explained by differences in stability and solubility. The evidence provided by Andrews, Schoen-

heimer, and Hrdina (31) and by Ravdin, Riegel, Johnston, and Morrison (52) to explain formation of calculi in the gallbladder receives additional confirmation from our data.

Other changes in chemical composition of bile in cholecystitis may in a large measure be referred to a primary loss of bile acid. Chloride or, less frequently, bicarbonate replaces the anion lost by diffusion of bile acid from the gallbladder. Thus the concentrations of these ions are increased. Diminished total solids and decreased concentration of total base likewise are directly related to the decline in bile acid concentration.

An end result of changes that occur in the contents of the gallbladder with an inert or abnormally functioning mucosa is replacement of the hydrolyzed salts of weak acids, i.e., sodium glycocholate and glycodesoxycholate, by sodium chloride, with a shift of pH from the slightly alkaline reaction normally observed toward more acid reactions. The distinctly acid reactions of many specimens from badly damaged gallbladders are unexplained, however, although loss of buffering capacity facilitates the change. Increased alkalinity, at times observed, results from secretion of bicarbonate under similar circumstances. Weiser and Gray (46) and others suggest as one mechanism for gallstone formation, a shift in pH from alkaline to acid with resultant precipitation of cholate. Actually, bile acid concentration governs the pH of bile. It is our belief that changes in pH are incidental to more fundamental changes in the constitution of bile, and that change of pH is of secondary importance as a causative factor in formation of cholesterol or mixed stones. Despite the primary rôle in cholelithiasis assigned to changes in pH by many workers, no suggestion has been offered by them as to how the hypothetical change in the reaction of bile giving rise to gallstone formation is to be brought about. Because of the acid reaction of many specimens of gallbladder bile, it is quite unlikely that soaps are present in significant amounts.

Calcium usually is lowered in the contents of diseased gallbladders, although this response to injury is variable. Occasional noteworthy exceptions with striking increases have been observed. While commonly lowered in comparison with val-

ues found in normal specimens, calcium concentrations remain higher than those of blood serum. It is significant that the concentration of bile calcium is lowered less in proportion to the normal than is the concentration of bile acid. Since bile acids undoubtedly aid in maintaining calcium (and bilirubin) in solution, the ubiquitous occurrence of calcium in gallstones would follow as a result of this altered relationship between bile acids and calcium. An additional factor that may favor precipitation of calcium is secretion of bicarbonate by the gallbladder. Although a typical response in dogs, our data suggest that it is infrequently encountered in humans. The behavior of magnesium differs from that of calcium, and magnesium concentrations are not maintained at high levels in the presence of gallbladder disease.

Sterile fluid found in an hepatic cyst closely resembled in composition the fluid collected from gallbladders where obliteration of functioning mucosa or complete long-standing obstruction of bile ducts had rendered the organ incapable of function.

Cholesterol crystals in the gallbladder contents quite consistently accompanied cholecystitis. The detection of such crystals in specimens of bile therefore provides good evidence for the existence of cholecystitis. Such evidence is not necessarily conclusive for occasionally crystals are found in the absence of pathological changes of the gallbladder. However, our data support the application of this test for diagnosis of cholecystitis, provided representative samples of gallbladder bile are secured for examination. A close correlation existing between the ratio of cholic acid to cholesterol and the incidence of cholesterol crystals or concretions reported by previous workers has been confirmed.

#### SUMMARY

Severe cholecystitis brings about serious defects in gallbladder physiology that result in marked alteration in chemical composition of gallbladder bile. Changes that have been observed in the presence of gallbladder disease are sufficiently marked (qualitatively and quantitatively) to lead to calculus formation. Cholesterol varies widely in concentration in the control specimens as well as in those specimens from pathological

gallbladders. Low values for cholesterol were found in the presence of obstruction of the bile ducts. Similarly, concentrations of bile pigment became lower progressively with increasing injury to the gallbladder.

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# CONCERNING THE NATURALLY OCCURRING PORPHYRINS. V. PORPHYRINS OF THE FECES<sup>1,2</sup>

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The complexity of the porphyrin problem is due in large part to the occurrence of spectroscopically identical isomers, those corresponding in configuration to hemoglobin or aetioporphyryn III, and those of the aetioporphyryn I type, whose formation is to be regarded as an independent synthesis. Recent work has demonstrated that porphyrins of the latter group are much more frequently encountered in the excreta. Coproporphyrin I was isolated from normal urine (1, 2, 3), and from the urine in increased amount in a variety of pathological conditions other than the idiopathic porphyrinurias (3, 4, 5). In earlier communications the writer described its isolation from the feces in hemolytic jaundice and pernicious anemia (6, 7). Coproporphyrin III has been encountered less frequently; as yet only in the urine in the following diseases: 1, Lead poisoning (3, 8); 2, exceptional instances of chronic porphyrinuria (9, 10); 3, the majority of instances of acute porphyrinuria (11, 12, 13, 14); 4, salvarsan treated individuals (15); 5, instances of hemochromatosis and atrophic cirrhosis (4). Schreus (16) has recently maintained that excretion of coproporphyrin III would be found to accompany increased blood destruction. This view is not supported by the fact that coproporphyrin I is excreted in urine and feces in hemolytic jaundice (3, 4, 6). As will be noted below, this finding is confirmed in the present investigation.

Since the reports of Snapper (17), Papendieck (18), Schumm (19, 20, 21), and Boas (22, 23, 24, 25), interest in the porphyrins of the feces has centered upon their significance in the detection of occult bleeding. The exact nature of the porphyrins derived from blood in the gastrointestinal tract was first demonstrated by Kämmerer (26), and Fischer and Lindner (27). These are protoporphyrin, deuterohemin, and

deuteroporphyrin. The last two are undoubtedly identical with the substances which Schumm named copratin and copratoporphyrin (19). The existing evidence concerning the derivation of coproporphyrin from blood in the intestinal tract is conflicting; Schumm (28) believed it to be the source of the coproporphyrin of the urine. However, Fischer and Schneller (29) demonstrated coproporphyrin in the urine and feces of vegetarians. The writer (30) isolated crystalline deuteroporphyrin IX, corresponding to hemoglobin, from the feces of a normal individual receiving meat in the diet. Coproporphyrin was not increased in this sample of feces although an increase might have been expected if one assumes that hemoglobin can give rise to coproporphyrin in the intestine. As will be noted below, this is borne out in the present investigation.

As yet the isomeric type of the coproporphyrin of normal feces has not been determined. If hemoglobin or meat were the source, coproporphyrin III would be expected. Kämmerer and Gürsching (31) found that many of the common foods, particularly those of plant origin, contain traces of coproporphyrin, which, by analogy to that formed in yeast cells, is probably coproporphyrin I. Fischer and Schneller (29) obtained crystals which were probably those of coproporphyrin I, from the feces of a vegetarian. Correlation of these findings suggests that the coproporphyrin of the feces is exogenous; it should be emphasized, however, that a great disproportion in amounts of porphyrin undoubtedly existed in these two studies, since it is certain that infinitely less porphyrin is necessary for the production of considerable fluorescence (the method of detection used by Kämmerer and Gürsching), than for the isolation of crystalline material. For this reason it is entirely possible that most of the porphyrin obtained by Fischer and Schneller was endogenous. Garrod (32) concluded that the bulk of the normal urinary and fecal porphyrin was endogenous. He pointed to the constant presence of porphyrin in the meconium. This

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was later identified by Schumm (28) as a coproporphyrin, and Waldenström (33) has recently made the important observation that it is coproporphyrin I. Fischer and Zerweck (34) likewise held the view that coproporphyrin is endogenous, and spoke of it as a normal product of metabolism. The occurrence of protoporphyrin in the erythroblasts of the marrow (35) and in the circulating erythrocytes (36) further supports the endogenous theory.

The porphyrin of human bile was identified by Schumm (28) as a coproporphyrin. Whether this is endogenous or whether absorbed from the bowel and re-excreted is not known. Borst and Königsdörffer (37) were unable to obtain experimental evidence for the direct reabsorption of porphyrins from the bowel. On the contrary, H. Fischer and Hilmer (38), and recently Brugsch (39) noted definite increases in urinary porphyrin after feeding small amounts of coproporphyrin. Although quantitative studies might solve the problem of the origin of coproporphyrins in the excreta, those so far reported (39, 40, 41) have dealt with only the total porphyrin excretion without attempt to separate proto-, deuterio-, and coproporphyrin. In addition to the necessity of determining them separately, it is evident that knowledge of the isomeric types excreted normally and in disease must be obtained prior to quantitative studies, since the latter cannot distinguish isomers.

The present investigation continues previous studies of the porphyrins in feces and urine; the scope of the investigation is as follows: (1) Isolation of porphyrins from (a) normal feces, (b) bile, (c), feces in lead poisoning, (d), urine in pernicious anemia, (e), feces in further instances of hemolytic jaundice and pernicious anemia. (2) Comparison of amounts of porphyrins in the feces in (a) pernicious anemia before, during, and after liver induced reticulocyte response, (b), normal individuals, patients with hemolytic jaundice and patients with jaundice due to complete common duct obstruction.

#### MATERIAL AND METHODS

##### *Group I*

*Case 1.* Normal. Male, 18 years of age. Eight day collection of feces. From the urine of this individual, collected for a longer, but contemporary period, copro-

porphyrin I was isolated, as described in Study IV (3).

*Case 2.* Normal. Male, 24 years of age. Four day collection of feces. Urobilinogen was 89.8 mgm. per day.

*Case 3.* Lead poisoning. Male, 46 years of age. Coproporphyrin III was isolated from the urine of this patient, as described in Study IV (3). The present collection of feces did not take place until four weeks after this isolation. In the interval there had been considerable improvement, and it was therefore necessary to re-examine the patient's urine as to porphyrin content. For this purpose the entire amount was collected during an eight day period of collection of feces. Urobilinogen in the feces was 173.4 mgm. per day.

*Case 4.* Hemolytic jaundice, familial. Male, 38 years of age. Eight day collection of feces. Patient had recurrent jaundice since infancy, and has known of enlarged spleen for many years. Father and one brother have jaundice and splenic enlargement. Hemoglobin was 56 per cent (Sahli; 17 grams per 100 cc. = 100 per cent), and average diameter of erythrocytes  $6.6\mu$ . There were many hyperchromatic microcytes in stained preparation of blood. The resistance of erythrocytes to hypotonic saline was  $H_1$ , 0.7 per cent,  $H_2$ , 0.46 per cent; control  $H_1$ , 0.44 per cent,  $H_2$ , 0.36 per cent. The icteric index was 18 and the Van den Bergh reaction on blood serum indirect. No bilirubin was demonstrated in the urine. Urobilinogen in feces was 2475 mgm. per day, in urine 2.6 mgm. per day. The normal range with the method (42) used is 40 to 280 mgm. and 0 to 4 mgm. per day respectively.

*Case 5.* Hemolytic jaundice, acquired. Female, 18 years of age. Eight day collection of feces. This case was described in Part IV ((3) Case 2), where the isolation of coproporphyrin I from the patient's urine was reported. The present study was made prior to the first operation. At this time the hemoglobin was 32 per cent, icteric index 42, feces and urine urobilinogen 1106 and 9.8 mgm. per day, respectively.

*Case 6.* Hemolytic jaundice, acquired. Female, 31 years of age. The clinical features in this instance are described elsewhere (43); for the present, it is sufficient to note that a hemolytic, macrocytic anemia appeared during the course of a long-enduring painless jaundice. Correlation with other manifestations of the disease, such as marked enlargement of the liver and spleen, marked bilirubinuria, and prompt Van den Bergh reaction of the blood serum indicated a diffuse affection of the liver. During the period of increased blood destruction and consequent regenerative anemia (the reticulocyte level attaining 15 per cent), urobilinogen excretion was greatly increased; the amounts in the feces ranged from 460 to 1250 mgm. per day during the two weeks in which the anemia developed. The urine urobilinogen at this time varied between 57 and 224 mgm. per day. During the first half of the present eight day period of collection of feces, the feces urobilinogen was 990 mgm. per day; in the second half 894 mgm. per day. The icteric index was 104, and, since bilirubinuria was prominent, it is



clear that normal bile flow had not yet returned. During the ensuing weeks, the jaundice gradually disappeared; five months from the time of onset the patient appeared to have recovered completely, although the spleen and liver were still palpable; at present she has remained well for nearly two years.

*Case 7.* Complete common duct obstruction. Male, 26 years of age. The patient had epigastric distress, hematemesis and melena of 3 years' duration. There was progressively deepening jaundice during the last two months of life. The urobilinogen excretion was of neoplastic obstructive type (43); feces: 0.3 mgm. per day; urine—tracc. Occult blood was present in the feces.

ogen ranged from 457 to 640 mgm. per day during the eight day period of urine collection prior to liver therapy.

*Case 8b.* Male 72 years of age. The patient complained of progressive weakness, pallor with slightly yellow skin, for 4 months. There was a smooth tongue. The hemoglobin was 28 per cent, erythrocytes 1,000,000 per cu. mm. Marked macro-anisocytosis and poikilocytosis were noted in the blood smear. The reticulocytes were 2.0 per cent. The feces urobilinogen was 803.6 mgm. per day during a six day period of collection of urine prior to liver therapy.

In each instance, the entire amount of urine

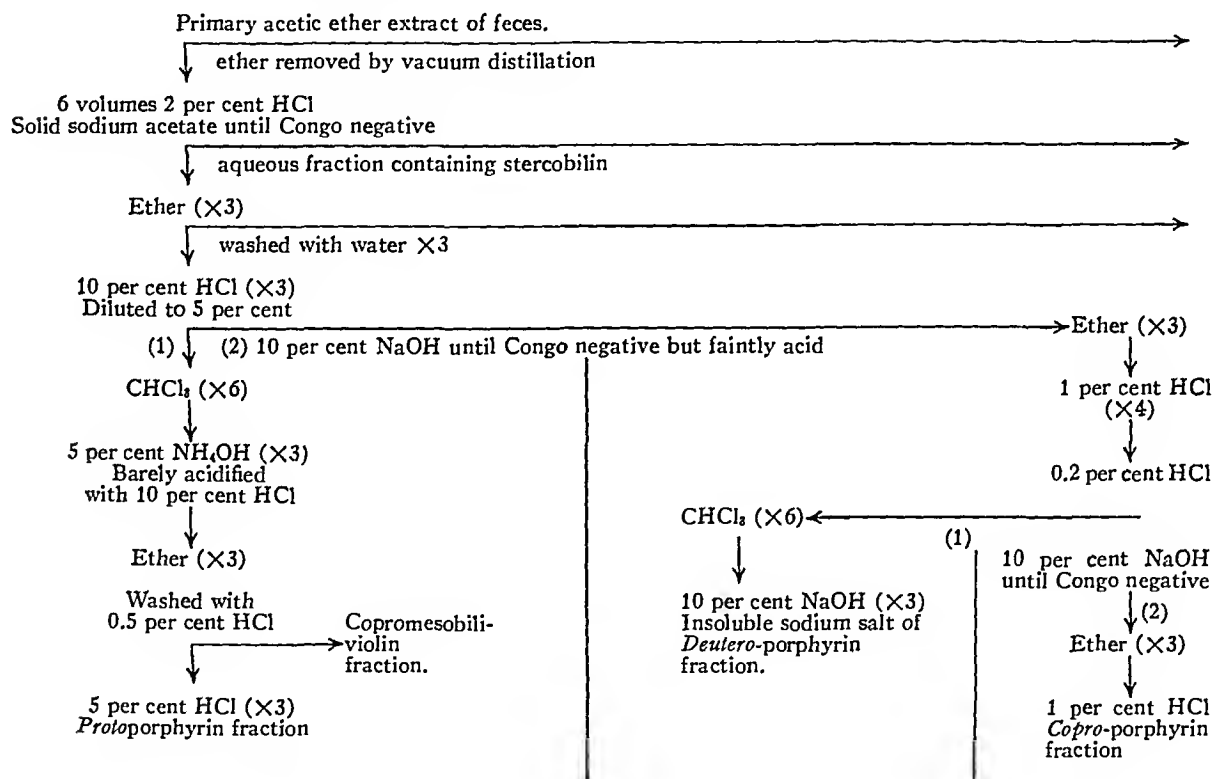


FIG. 1. MODIFIED FRACTIONATION OF PORPHYRINS OF FECES AS EMPLOYED IN CASES OF GROUP 2.

Necropsy on October 28, 1935, revealed a diffuse scirrhous carcinoma of the stomach of linitis plastica type, with marked diffuse involvement of the duodenum and common bile duct. Eight day collection of feces, October 11 to October 19.

*Case 8a.* Pernicious anemia. Urine. Male, 55 years of age. Progressive weakness of legs had been noted for 3 years, paralysis for 1 year. There was definite evidence of combined degeneration of the spinal cord. Slight icterus and smooth tongue were observed. The hemoglobin was 38 per cent, erythrocytes were 1,400,000 per cu. mm. Marked macro-anisocytosis and poikilocytosis were seen. The reticulocytes were 1.0 per cent. Achlorhydria gastrica was present. The feces urobilin-

was subjected to the procedure described in Studies I and IV (3, 5). The coproporphyrin obtained was in such small amount that it was clearly necessary to combine the final solutions from the two cases in order to isolate sufficient crystalline material to determine the melting point. This combination was less objectionable in view of the close clinical similarity of the two patients, in both of whom the findings were typical of pernicious anemia in relapse. Feces in the above instances were subjected to the isolation procedure described in Study II (6).



superimposed mesoporphyrin IX ester: I 621.7, II 574.2, III 533.0, IV 500.0. (The mesoporphyrin dimethyl ester employed for this comparison was obtained from hemin by Fischer and Kögl's method (44) and subsequent esterification by the usual HCl-methyl alcohol procedure; melting point 212° C.)

As already noted, a relatively considerable amount of protoporphyrin was obtained in Case 4 of Group II (hemolytic jaundice). Two weeks elapsed, from the time of the above mentioned spectrometric comparisons with the protoporphyrin of normal feces, until further study could be carried out. In this interval, although the 5 per cent HCl solution was most of the time in the refrigerator, it was evident that a change had occurred. Most of the porphyrin now present was no longer a protoporphyrin but had characteristics more nearly those of a deuteroporphyrin. The majority was now extractible from ether with 1 per cent HCl (absorption: I 592.7, II 548.9) while a small proportion, having a more bluish color in dilute HCl, still behaved like a protoporphyrin in that it was not extracted from ether with 1 per cent HCl, and in 5 per cent HCl exhibited a protoporphyrin absorption spectrum, i.e., I 594.8, II 556.0. The porphyrin extractible with 1 per cent HCl had the following absorption in ether and acetic: I 624.0, II 577.4, III 531.6, IV 498.5. In 10 per cent NaOH the sodium salt was almost entirely insoluble. Further mention of this porphyrin will be deferred until a description has been made of a similar deuteroporphyrin-like substance which occurred in several instances.

Particular attention was given to the following deuteroporphyrin fractions. Group I, Cases 1, 4, and 7; Group II, Cases 2a and b, 3a, b, and c, 4, 5, and 6. In the other cases of both groups, the amount present was too small to permit spectroscopic identification. Deuteroporphyrin was encountered in Case 7 of Group I, and in Cases 2b and 6 in Group II. In the first and last of these three instances its presence was accounted for by bleeding; the relatively small amount present in the feces of Case 2b, Group II, may have been due to meat in the diet. It is of considerable interest that the same fraction in the other cases contained a porphyrin with an absorption spectrum differing from deuteroporphyrin, but quite

identical with that of the porphyrin described in Study III (7) whose ester crystallized in long prisms melting at 189 to 191° C. For the time being at least this will be designated as pseudo-deuteroporphyrin A. In the present investigation it has been encountered in six instances, i.e., in all of the above except Cases 2a and b of Group II. In two of these, crystals of the methyl ester were obtained; these were noted as having the same form illustrated in Study III (p. 118 (7)); in neither was the amount sufficient for melting point determinations. In each of the six instances the absorption spectrum was identical (by superimposition) with that of the porphyrin described in Study III: in ether I 623.8, II 575.5 (midpoint of absorption band; maximum intensity at 568.0), III 528.7, IV 496.3. In 1 per cent HCl, I 590.4, II 547.6.

In each instance superimposition of absorption spectra was carried out with solutions of known copro-, hemato-, and deuteroporphyrin. The absorption differed only slightly from that of copro-, and more sharply from hemato- and deuteroporphyrin. On the other hand, the sodium salt of this porphyrin is insoluble, its HCl number is 0.3 to 0.4, and it is extractible from 0.2 per cent HCl with chloroform, characteristics which distinguish it sharply from coproporphyrin and which are responsible for its designation as a pseudo-deuteroporphyrin. In Case 3 of Group II, the amounts in the three periods, a, b, and c, were in an approximate proportion of 4 to 6 to 1, respectively. It has already been noted that the ratio of amounts of copro- and protoporphyrins for the three periods was essentially the same as this.

In the above it was noted that the protoporphyrin fraction of Case 4 (Group II) after standing for two weeks, contained only an inconsiderable amount of protoporphyrin; most of the porphyrin present now had the characteristics of pseudo-deuteroporphyrin A. The substance was readily extracted from ether with 1 per cent HCl, and after dilution to 0.2 per cent HCl it was in turn extracted by chloroform. In 10 per cent NaOH the sodium salt quickly precipitated. The absorption spectrum was as follows: In ether and acetic acid, I 623.3, II 596.8 (faint), III 575.3, IV 528.8, V 496.0; in 1 per cent HCl, I 588.8, II

547.3. This was entirely identical with pseudo-deuteroporphyrin A, when the absorption spectra were superimposed. A very slight difference was noted when superimposed with copro- and a greater difference when with deuteroporphyrin.

In Study II, a new porphyrin, also encountered in the deuteroporphyrin fraction, was found in the feces of a patient with hemolytic jaundice. The methyl ester of this porphyrin crystallized in flower-like aggregates and melted at 202° C.; spectroscopically it was characterized by absorption bands about midway between those of proto- and copro- or deuteroporphyrin. Thus the maximum of the redward band was at 627 to 629  $m\mu$ . In view of the exceptional broadness of this band, Professor H. Fischer considered the possibility of a molecular compound between two porphyrins, such as proto- and deuteroporphyrin. Because of the limited amount of material this question could not be decided. Later, a porphyrin with the same solubilities and absorption spectrum, but in an amount insufficient for isolation, was noted in the feces of another patient with hemolytic jaundice. In the present investigation a porphyrin of the same type was encountered in Case 3a (Group II), an instance of pernicious anemia in relapse. When the absorption spectrum of this porphyrin was superimposed with that of the porphyrin from the earlier investigation (ester melting point 202° C.), complete identity was noted. In view of the fact that this also behaves like a deuteroporphyrin in many respects, it may be designated as pseudodeuteroporphyrin B (pending more exact information as to its chemical structure).

#### DISCUSSION

In the present investigation coproporphyrin I has been isolated repeatedly from normal feces and in one instance from fistula bile. It was absent from the feces of two patients having complete biliary obstruction; this lends support to the belief that the substance is chiefly endogenous, especially when correlated with the marked increase again observed in hemolytic jaundice.

Since coproporphyrin I is not a derivative of hemoglobin, but is rather the product of an independent synthesis, and since the amounts excreted are greatest in instances where regeneration and bone marrow activity are most marked, it has be-

come more and more evident that the excretion of coproporphyrin I in the feces, at least in patients with normal liver function and without biliary obstruction, is related to erythropoietic activity. There is much reason to believe that the coproporphyrin I of bile and feces is derived from the protoporphyrin which Van den Bergh and Hyman (36) found to occur in a very small amount in the circulating erythrocytes, and which Borst and Königsdörffer (35) had earlier observed in marrow erythroblasts. Van den Bergh et al. (47) first suggested this relationship after demonstrating that the surviving liver is capable of converting proto- into coproporphyrin. The amount of protoporphyrin in the normal erythrocytes is obviously very small; it is unlikely that even a sudden destruction of a majority of the erythrocytes would provide the amounts of porphyrin encountered in hemolytic jaundice feces. This is probably illustrated by the present case of paroxysmal hemoglobinuria in which intravascular hemolysis was sufficient to reduce the hemoglobin rapidly to 22 per cent; in spite of this, relatively little coproporphyrin was found in the feces, and although considerable protoporphyrin was also present, the total porphyrin did not compare in amount with that seen in hemolytic jaundice. The difference seemed too great to be explained by the disparity of ages. In this patient, however, the period<sup>7</sup> of collection of feces followed immediately upon the first hemolysis, and probably preceded any appreciable increase of hemopoietic activity particularly when the time required for traversal of the intestinal tract is taken into account. By contrast, hemopoietic activity is quite constantly increased in hemolytic jaundice, so that the feces of any interval are certain to represent a period of heightened erythrocyte metabolism.

The above considerations focused the writer's attention upon the possibility of relationship between the erythrocyte-protoporphyrin and the reticulated cells. It has in fact been shown (48) that most if not all of this protoporphyrin resides in the reticulocytes, not in the mature erythrocytes. It is quite probable, therefore, that much larger amounts of protoporphyrin are available in blood containing a large number of maturing reticulocytes.

<sup>7</sup> Opportunity for investigating feces of subsequent periods was not afforded in this instance.

If the protoporphyrin of the reticulocytes is the parent substance of the coproporphyrin I found in bile and feces, then it is highly probable that it likewise has the configuration of aetioporphyrin I. Otherwise one would have to assume that the protoporphyrin underwent destruction to its component pyrrol nuclei with subsequent re-synthesis of coproporphyrin I. H. Fischer (14) believes a transformation of this type to be extremely unlikely.

In the present investigation it was usually noted that protoporphyrin was increased in the same instance where the feces contained definite increases of coproporphyrin I. The only exceptions were the two patients with total biliary obstruction, whose feces contained no copro- but considerable proto- and deuteroporphyrin, the occurrence of which was readily explained by the presence of blood. Of greater interest was the protoporphyrin found in feces which did not contain demonstrable occult blood, i.e., in instances of hemolytic jaundice, pernicious anemia, paroxysmal hemoglobinuria. Attempts to identify this protoporphyrin with certainty were unsuccessful; nevertheless its behavior, as well as the behavior of the mesoporphyrin obtained from it, suggested that it differed from protoporphyrin as obtained from hemoglobin. In the case of paroxysmal hemoglobinuria, the amount of protoporphyrin was relatively large, unquestionably exceeding that of the coproporphyrin. In this instance, as in the others where an attempt was made to isolate the substance, it was evident that constant deterioration occurred during the various fractionations.

In the cases of pernicious anemia who were followed through the period of reticulocyte response, it was noted that the excretion of coproporphyrin I in the feces increased slightly over that observed during relapse; after the reticulocyte response, a considerable diminution occurred. This again points to a relationship between erythropoietic activity and excretion of coproporphyrin I. The increases were not as great as one would have expected if the circulating reticulocytes were assumed to be the sole source of porphyrin. However, there is no reason to doubt that porphyrin may be furnished directly from the megaloblasts in the pernicious anemia marrow (37); whether

this is dependent upon release occurring with maturation, or due to the phagocytic destruction which Peabody and Broun (49) described, remains to be determined.

The significance of the two "pseudodeuteroporphyrins" is not clear. Certain evidence, already mentioned, suggests that they are likewise derivatives of protoporphyrin. The spectroscopic character, chloroform solubility, HCl-number, and insoluble sodium salt of pseudodeuteroporphyrin A suggest similarity with the porphyrin which Schummi (50) described under the name "sapro-porphyrin."

It is probable that a porphyrin which the writer noted (30) in increased amount in the feces of a patient with hemolytic jaundice, which was believed then to represent deuteroporphyrin, was in reality pseudodeuteroporphyrin A.

Borst and Königsdörffer (37) observed a porphyrin in fetal liver, and in the liver of the famous case Petry (congenital porphyrinuria, pernicious anemia) which they designated as 627 (maximum absorption of redward band at that wavelength). Their studies indicated that this was derived from protoporphyrin, and they evidently considered the possibility that it might be a transition between proto- and coproporphyrin. The similarity of absorption between this porphyrin 627, and the above mentioned pseudodeuteroporphyrin B suggests a close relationship or possible identity.

The isolation of coproporphyrin I from the feces of the patient with lead poisoning, during a period in which the urine contained coproporphyrin III, emphasizes that one may not draw conclusions about the type of urinary porphyrin on the basis of the isomer present in the feces. Coproporphyrin I had been isolated repeatedly from the feces of patients with pernicious anemia, but the type in the urine had not been identified. The porphyrin isolated in the present study from the urine of two typical cases during relapse, proved to be coproporphyrin I.

The findings in the present instance of lead poisoning suggest that coproporphyrin III is either eliminated less easily by the liver, or is re-absorbed to a greater degree from the bowel than is coproporphyrin I. It is quite possible that coproporphyrin III will be found in the feces of

a more severe or more acute case than was represented in this study.

The results of the present investigation support the contention of Boas (22) that protoporphyrin in the feces does not originate solely from ingested hemoglobin nor from occult bleeding, but is also "physiological." Boas noted that it was still demonstrable in feces of a normal individual even after a long period on a milk and vegetable diet. The present study reveals that it is often increased in association with increases of coproporphyrin I in the feces of patients with heightened hemoglobin metabolism. Corresponding increases of deuteroporphyrin were not observed; in agreement with Boas (23) it is believed that this porphyrin owes its formation solely to the putrefaction of hemoglobin in the bowel. It is important to emphasize, however, that deuteroporphyrin may be confused readily with pseudodeuteroporphyrin A except when considerable purification and an exact spectrometric study have been resorted to. Because of this, the usefulness of the deuteroporphyrin test for occult bleeding is distinctly reduced. In some instances of persistent occult bleeding due to gastric carcinoma, Boas (25) noted an increased amount of coproporphyrin. The isomer type was not identified, but it is unlikely that this was other than coproporphyrin I. Since many individuals with persistent occult bleeding exhibit elevation of reticulocytes, the increased coproporphyrin observed by Boas may have been due to increased hemopoietic activity.

#### SUMMARY AND CONCLUSIONS

1. Coproporphyrin I has been isolated from normal human feces, and from human fistula bile. It was not found in the blood-containing feces of two patients having gastro-intestinal neoplasms, who also suffered from complete biliary obstruction. Considerable increases of coproporphyrin I were again noted in feces from patients with hemolytic jaundice. Correlation of these findings indicates that coproporphyrin I is chiefly endogenous. That it may be derived from the protoporphyrin of the circulating reticulocytes or from that of immature erythrocytes in the bone marrow, is a possibility which must be considered.

2. Increases of protoporphyrin were frequently encountered in association with increased copro-

porphyrin I in feces not containing demonstrable occult blood. Certain differences in behavior suggest that the isomer type of this protoporphyrin is not the same as that derived from hemoglobin.

3. Coproporphyrin III was isolated from the urine of a patient recovering from lead poisoning; the feces for the same period contained coproporphyrin I. Thus it is clear that conclusions about the isomer type of a coproporphyrin occurring in increased amount in the urine may not be based upon the type found in the feces.

4. Coproporphyrin I was isolated from the mixed extracts of urine of two patients with pernicious anemia, in relapse.

5. Tests for occult blood in the feces which are based upon the presence of porphyrin are of doubtful value unless a careful fractionation is carried out, with subsequent spectrometric identification. In particular, the pseudodeuteroporphyrins described here must be distinguished; their behavior is very similar to that of deuteroporphyrin, but their origin has not been established.

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# PATHOLOGY OF PREGNANCY TOXEMIAS

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In an attempt to determine the cause and nature of the toxemias of pregnancy, the records of patients who were admitted to the New Haven Hospital with the diagnosis of toxemia of pregnancy or who had renal or vascular disease that had apparently arisen during pregnancy have been reviewed. Altogether 309 of the first class and 11 of the second were found with records complete enough to warrant analysis. Of this group 53 died and 25 came to autopsy. The present paper deals with 23 of these autopsies. One of the 25 was omitted because the data of the autopsy, which was performed at another hospital, are inadequate; the second because there is some doubt about the diagnosis of toxemia. This patient had a diaphragmatic hernia which was responsible for her death and possibly for the earlier symptoms that were ascribed to toxemia.

The essential *postmortem* findings and clinical data are presented at the end of the article. In 10 instances, only the briefest clinical notes are given, because these cases have been reported in detail in another connection elsewhere (1). A brief tabulation of the patients appears in Table I. Thirteen died as a direct result of the toxemia, which took an eclamptic form in 6; the other 10 died with late sequellae of the disease, which had begun with an eclamptic attack in one.

The first notable thing is the frequency with which pyelitis or its ultimate consequence, pyelonephrosis, is encountered. In this series these conditions were found in 10 (possibly 11) of the 23 subjects, 3 (possibly 4) with acute deaths and 7 among the remote chronic deaths. One of the latter, 63494, initially had typical eclampsia and 2 of those who died acutely. A39242 and A1714, also had eclampsia. The third, who died acutely, 3666, might also be placed in the eclamptic class, save for the absence of convulsions. Objection may be raised to the application of the terms toxemia or eclampsia to patients who have obvious pyelitis. The strongest justification for such a course lies in the fact that they received these diagnoses during life because they presented clinical signs and symptoms indistinguishable from those which characterize the toxemias. In fact, the true pathological lesions in none of these cases were surmised before autopsy. This is also true of most of the chronic cases, which were believed to have glomerular nephritis or nephrosclerosis.

If the three acute cases, A39242, A1714 and 3666, are analyzed further, it is found that they present, in addition to pyelitis and pyelonephritis, certain more subtle pathological lesions which are quite widely accepted as characteristic of eclampsia. These deserve some description and discussion before the argument proceeds.

Degenerative changes and necrosis of the epithelial cells of the renal tubules have long been recognized as the commonest pathological lesions of the toxemias of pregnancy. By some, they have been connected more particularly with the convulsive syndromes to which the term eclampsia has been applied. The degree of degeneration of the tubular cells varies from mere cloudy swelling to a necrosis which may be so extreme and widespread that the epithelium seems to be almost as universally destroyed as it is in acute mercurial poisoning. The process is usually maximal in the convoluted tubules. Because the lesion is

TABLE I  
*Resumé of causes of death*

	Total	Eclampsia
Acute deaths (directly referable to toxemias) .....	13	6
Pyelitis—A39242* A1714, 3666 (A32601) † .....	3	2
Arterial—79045, 83867 .....	2	
Acute eclampsia—81551, 61702, 9354, (60242) ‡ .....	4	4
Infectious—A32601, 71013, 18925, 33564 .....	4	
Deaths from late sequellae .....	10	1
Pyelonephritis—63494, 44154, 53431, 31841, 8250, 47162, A9526 .....	7	1
Arterial—20067, 43495, 58750 .....	3	

\* Eclamptic cases in italics.

† This patient had one ureter dilated and thickened.

‡ Convulsions only in agonal state.



purely desquamative Volhard and Fahr (2) have identified it with nephrosis.

In addition to the tubular lesions definite changes can be discerned in the glomeruli. These have been minutely described by Bell (3), who considers them more characteristic than the tubular lesions. The lining membrane cells occasionally become swollen, sometimes necrotic—when they may be desquamated; the endothelial cells of the capillary loops more frequently are also swollen. They tend to increase in number and impart an excessive cellularity to the glomeruli. The lumina of isolated capillary loops appear to be occluded by swollen and proliferated endothelial cells. The basement membrane between the lining epithelium and capillary endothelium becomes thickened, in consequence of which the capillary loops, especially where they appear in cross section, have a staring open appearance with sharp double outlines to their walls. This change in the basement membrane is perhaps the most characteristic feature of the renal lesions in the toxemias. It may be so marked as to obliterate the capillary lumen. In addition, material resembling fibrin in its staining reactions appears in localized deposits that may be quite diffuse in certain capillary tufts. The glomeruli usually appear rather avascular, although there is distinct variability in this respect. Not rarely congested loops are seen, and extravasated blood cells may appear in the glomerular spaces. These are, however, more often found empty or contain only a little albuminous material and occasional desquamated epithelial cells. Scattered tubules, likewise, may contain masses of blood; but the great majority are filled with albumin and cellular debris. Often the narrow lumen left by the swollen epithelium appears to be entirely plugged by this debris. The striking thing about such kidneys is the absence of inflammatory reaction in the interstitial tissue and the preservation of those structures which are essential for the architecture of the organs. One derives the impression that no damage has been done which is not reversible or reparable, like the comparable injury of mercurial poisoning.

Whether the condition arises from primary vascular injury or whether it affects the renal epithelium directly, it is not easy to say. Post-

mortem examination reveals no similar lesions elsewhere in the vasculature or in other organs except the liver, which will be given especial consideration later. In certain instances, to be sure, focal hemorrhages may be found in the heart, and other organs, and both edema and hemorrhages are quite regularly found in the retina. However, the possibility of generalized vascular changes cannot be summarily dismissed because it cannot be demonstrated in autopsy material. The invariable incidence of hypertension is incontrovertible clinical evidence of a generalized vascular reaction, whether this be merely a functional response or connected with anatomical changes that have thus far escaped detection.

Renal lesions are so invariably found in patients who died of acute toxemias that there can be little doubt of their significance. Moreover, the symptomatology of the toxemias resembles more than anything else the picture of acute nephritis. Edema, hypertension, albuminuria and convulsions, with or without visual disturbances, clearly spell nephritis to anyone with experience in clinical medicine. Nevertheless, great importance has been attached to certain lesions in the liver which are encountered in a portion of patients with toxemias. These lesions in their most typical form consist of hemorrhages and necrosis of the hepatic cells, especially in the periportal areas. By some, these are attributed to the presence of fibrin or hyalin thrombi in the periportal venules. It is generally admitted that these lesions are less consistently found than those of the kidney, and furthermore that the symptoms of toxemias do not resemble those of hepatic disease. Nevertheless, most extensive investigations have been carried out in an attempt to incriminate the liver. It is true that some measure of hepatic insufficiency can be demonstrated in many patients with severe toxemias and that jaundice is not uncommon in these conditions. A certain number of women, moreover, in the later stages of pregnancy or in the puerperium develop jaundice without any of the classical signs of toxemia. They are presumably suffering from destructive lesions of the liver which may be so extreme as to produce the picture of fatal acute yellow atrophy. Whether this is merely an advanced stage of the periportal necrosis of "eclampsia" cannot

be stated with certainty. There is no physiological nor pathological evidence that hepatic destruction can give rise to hypertension. Consequently, there has been a growing tendency to relegate the liver lesions to a subordinate place in the etiology of toxemic symptoms, and to concede the chief rôle to the kidney.

If it be granted that the ultimate criteria of toxemias, and especially eclampsia, are not clinical signs and symptoms, but the characteristic pathological lesions, the three acute pyelitis cases

neither presented at any time symptoms or signs which could be called eclamptic. Both died from rupture of dissecting aortic aneurysms and presented vascular lesions in kidneys and other organs that conform to the description of malignant nephrosclerosis, according to Volhard and Fahr (2). Both had received a diagnosis of arterial hypertension on the basis of the symptoms and signs during their initial toxemias, the last pregnancies and the intervening periods. The blood pressure in Case 83867 was so variable and

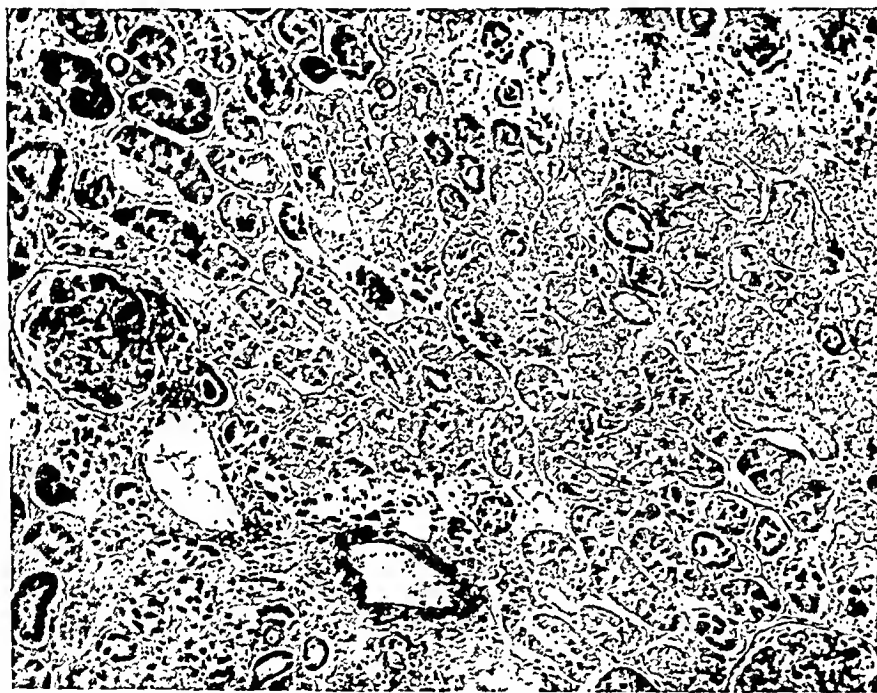


FIG. 1. CASE A39242. PYELITIS EARLY IN FIRST PREGNANCY CULMINATING IN ECLAMPSIA.

Note extensive tubular epithelial necrosis. Hematoxylin-eosin stain;  $\times 125$ .

(A39242, A1714, 3656) cannot be excluded (Figures 1 to 4). All exhibited typical tubular and glomerular changes, and one, at least, A39242, also had periportal necroses. To argue that the relation between pyelitis and toxemia in these cases was merely coincidental involves a dangerous preconception. It may well be that these renal and hepatic lesions represent only the pattern in which the pregnant woman reacts to a variety of insults. Some support for such an hypothesis is found in Cases 79045 and 83867. Both of these patients died during recurrent tox-

her symptoms and signs so slight that she was considered to have benign or functional nephrosclerosis. In the light of these facts the *post-mortem* anatomical findings are surprising in the extreme. The existence of arterial disease of a malignant type is undeniable. That it antedated the final pregnancy is highly probable in view of the history and physical findings. Nevertheless, the kidneys and livers of both patients had characteristic "eclamptic" lesions. In fact Case 83867 probably presents the most extreme periportal necroses found in this series of autopsies.



endothelium, so marked in some instances as to obliterate completely the capillary lumina (Figure 2). In some, massive deposits of pink-staining, fibrin-like material replaced entire capillary tufts and produced an appearance similar to that seen in so-called malignant nephrosclerosis (Figure 3). An occasional glomerular space contained desquamated epithelial cells. In the right kidney there were small scars in the cortex and a few hyalinized glomeruli which were attributable to the hydronephrosis. No definite vascular lesions were found in either kidney.

The architecture of the liver was altered by a distortion of the cellular columns, which was most marked in the periportal zones. The cells of these columns contained granular cytoplasm and vacuoles. Sometimes they were entirely necrotic in rather wide areas not strictly limited to the periphery of the liver lobules. There were no associated hemorrhages, and the periportal vessels were not thrombosed.

#### Case A1714

(For details of history see (1), Case 2.) During first pregnancy in 1929 in bed 7 weeks before delivery with edema. Near the end of second pregnancy, in 1931, *eclampsia*, after 10 days of extreme urinary frequency and 7 days of edema. Died within 24 hours.

*Necropsy.* The uterus was enlarged to the size of an eight months' pregnancy. The fetus occupied a normal position with the head at the cervix. The placenta was attached normally at the fundus of the uterus.

The kidneys, which weighed 250 and 275 grams respectively, had a homogeneous brown color externally, and their cortices were increased in width to 10 mm. Small hemorrhages were present beneath the epithelial lining in several of the renal calices. All the calices, the renal pelvis and ureters but particularly those on the right, were dilated. The dilatation began at the pelvic brim bilaterally.

The liver weighed 2400 grams, was firm in consistence and had externally a mottled hemorrhagic appearance. When the liver was sectioned, the hemorrhages appeared irregular in outline and varied greatly in size, obscuring the lobulation.

*Microscopic.* Small numbers of leukocytes were present beneath the epithelium of the renal pelvis. The interstitial tissue of the pyramids was edematous and infiltrated with small numbers of leukocytes and lymphocytes. Occasionally, the lumina of the straight tubules contained casts of cellular debris, amorphous granular material and polymorphonuclear leukocytes. Throughout the kidney, in the epithelium lining the convoluted tubules, there was a degenerative change, which consisted of cloudy swelling and granulation of the cytoplasm. Desquamation of these cells, however, was not extensive. All the glomeruli showed mild to moderate thickening of the basement membrane between capillary endothelium and lining epithelium. Most glomeruli appeared anemic, but in a few there were hemorrhages, sometimes extending into the glomerular spaces and appertaining convoluted tubules. Capillary tufts in occasional glomeruli seemed to be replaced by fibrin masses. The afferent arterioles and other

vessels of arteriolar size had greatly thickened, acellular walls.

In the liver were numerous focal hemorrhages, chiefly in the vicinity of periportal zones. These varied in size from a few extravasated red cells to masses which involved several contiguous lobules. Necrosis of liver cells accompanied the hemorrhagic lesions. Fragmented cells were seen in the interiors of leukocytes and large mononuclear cells. Large collections of lymphocytes were present around the periportal vessels, but none of the latter were thrombosed.

#### Case 3666

(For details of history see (1), Case 3.) In 1919 second pregnancy terminated at 8 months for toxemia. In July, 1921, miscarriage at 5 months, at which time there was distinct edema. In November, 1921, during third pregnancy, developed cold in chest with cough, followed a few days later by chills, dyspnea, orthopnea, headache, vomiting, pains in chest, limbs and back, and blurred vision. The condition became worse steadily until death 4 weeks after the onset of symptoms.

*Necropsy.* Both kidneys were small, the right weighing 55 grams and the left 31 grams. Their external surfaces were irregular and scarred. The pelves were greatly dilated; the cortex and pyramids were compressed to form a rim only 5 to 10 mm. in width. Cortical striations could be seen not at all or only with great difficulty. Both ureters were dilated, but the urinary bladder was small and contracted; its wall measured 12 mm. in thickness. The entire urinary tract was filled with thick, purulent material. The mucosa of the pelves, ureters and bladder was thick, opaque and contained numerous small hemorrhages.

The heart was increased in size, weighing 300 grams (body weight 38 kilos), and the papillary muscles were hypertrophied. There was no evidence of chronic passive congestion of the viscera and no edema nor ascites.

*Microscopic.* Extensive scars infiltrated with numerous lymphocytes and plasma cells replaced much of the renal parenchyma. Enormously dilated tubules with flattened epithelial cells and coagulated material in their lumina alternated with atrophic tubules. The majority of the glomeruli were replaced by densely hyalinized connective tissue; a few, less severely injured, had thickened capsules and an occasional adhesion between tuft and capsule. Both arteries and arterioles had concentrically thickened walls which reduced the size of their lumina.

The mucosa of the pelves, ureters and bladder was much thickened in some places. In others it was necrotic or desquamated and replaced by chronic granulation tissue containing numerous capillaries, lymphocytes and plasma cells which extended into the submucosa (Figure 4). A hemorrhagic exudate was seen on the free surface.

#### Case 79045

Born 1899. Patient is reported to have been entirely well during pregnancies in 1915, 1918, 1920, 1923, 1926 and 1927. All except the last, which ended in a pre-

rangement has been discovered. The changes in the arterioles, like those in the renal parenchyma, in mild or early cases would have to be entirely reversible and therefore could not be profound. Such should be the *Anlage* of the lesions of malignant nephrosclerosis. If this argument is reasonably sound, the changes in the kidneys, liver and vessels in toxemias should be looked upon as tissue responses to some general stimulus, comparable to the reactions of rheumatic fever or glomerular nephritis. Possibly in many cases the analogy can be carried further; the stimulus which elicits the response may be an infection, acting not directly upon the kidney by bacterial invasion, but invoking the tissue reaction from some remote local focus. This hypothesis is not inconsistent with the nature of the lesions of malignant nephrosclerosis which, even when fully developed, suggest degeneration far more than they do inflammation.

It is not intended to imply that there is any exclusive relation between toxemias of pregnancy and malignant nephrosclerosis. This would be absurd since the latter disease draws no sharp sex line. Pregnancy is pictured throughout merely as a state of temporary physiological disequilibrium which determines a predisposition to certain pathological conditions and, when these have developed, lends them a distinctive coloration. Plausible explanations can be found for some features of the predilection and the distinctive coloration. Physiological hydronephrosis, for example, obviously predisposes to pyelitis and prevents proper drainage and elimination of infection. Physiological hypoproteinemia makes albuminuria peculiarly vicious and enhances the tendency to edema. Other disturbances of equilibrium could be cited, such as the reduction of serum sodium; but little can be gained as yet by pursuing the subject further along these lines because sufficient facts have not yet been collected to complete the story.

#### SUMMARY AND CONCLUSIONS

1. Case histories and necropsy findings on 23 patients who died as a result of the direct or remote effects of pregnancy toxemias are reported.

2. Although characteristic tubular and glomerular lesions were usually found in the kidneys of patients who died in the acute stages of toxemias

with eclamptic syndromes, these lesions, like hepatic necroses, were not found exclusively in eclampsia.

3. Eclampsia could not be distinguished from other toxemias on the basis of either pathogenesis or morbid anatomy.

4. Lesions of "malignant nephrosclerosis" were encountered with frequency.

5. Both acute nephritis of infectious origin and antecedent vascular disease apparently gave rise to toxemias, which sometimes had eclamptic manifestations.

6. A great variety of vascular or renal diseases may act as predisposing causes for toxemias; pregnancy appears to give them a distinctive coloration and an explosive character.

#### PROTOCOLS

##### Case A39242

(For details of history see (1), Case 1.) Pyelitis appeared early in the first pregnancy, which culminated, during delivery, in *eclampsia* which proved rapidly fatal.

*Necropsy.* The uterus rose 14.5 cm. above the symphysis pubis in the midline. It was boggy to palpation and was covered by a smooth, glistening peritoneal surface. On section the endometrium was quite red; there was no retained placental tissue.

The right kidney weighed 175, the left 170 grams, both presenting an identical appearance. The renal pelves were considerably dilated and encroached to a moderate degree upon the parenchyma of the organs. The calices were irregular in size and shape and distinctly enlarged. Both ureters were dilated, beginning at a point opposite the lower poles of their respective kidneys. The dilatation of ureters, pelves and calices was more marked on the right side than on the left. The renal cortex was narrow and its architecture blurred.

The liver was a large flabby organ weighing 1933 grams. Scattered over all its surfaces externally and on section were numerous irregular pale yellow patches of softer consistency and greater friability than the adjacent tissue.

*Microscopic.* The epithelium lining the renal pelvis and the parenchyma beneath was infiltrated over wide zones with lymphocytes, plasma cells, eosinophilic and neutrophilic leukocytes and large mononuclear cells. Clusters of similar cells were found in the interstitial tissue of the pyramids and cortex. The tubular epithelium showed extensive degenerative lesions, most marked in the convoluted tubules, which varied from simple cloudy swelling to complete necrosis and desquamation (Figure 1). On the whole the glomeruli were anemic, but wide variations were encountered in this respect. Many seemed to be increased in size. In nearly all there was thickening of the basement membrane of the capillary

endothelium, so marked in some instances as to obliterate completely the capillary lumina (Figure 2). In some, massive deposits of pink-staining, fibrin-like material replaced entire capillary tufts and produced an appearance similar to that seen in so-called malignant nephrosclerosis (Figure 3). An occasional glomerular space contained desquamated epithelial cells. In the right kidney there were small scars in the cortex and a few hyalinized glomeruli which were attributable to the hydronephrosis. No definite vascular lesions were found in either kidney.

The architecture of the liver was altered by a distortion of the cellular columns, which was most marked in the periportal zones. The cells of these columns contained granular cytoplasm and vacuoles. Sometimes they were entirely necrotic in rather wide areas not strictly limited to the periphery of the liver lobules. There were no associated hemorrhages, and the periportal vessels were not thrombosed.

#### Case A1714

(For details of history see (1), Case 2.) During first pregnancy in 1929 in bed 7 weeks before delivery with edema. Near the end of second pregnancy, in 1931, *eclampsia*, after 10 days of extreme urinary frequency and 7 days of edema. Died within 24 hours.

*Necropsy.* The uterus was enlarged to the size of an eight months' pregnancy. The fetus occupied a normal position with the head at the cervix. The placenta was attached normally at the fundus of the uterus.

The kidneys, which weighed 250 and 275 grams respectively, had a homogeneous brown color externally, and their cortices were increased in width to 10 mm. Small hemorrhages were present beneath the epithelial lining in several of the renal calices. All the calices, the renal pelves and ureters but particularly those on the right, were dilated. The dilatation began at the pelvic brim bilaterally.

The liver weighed 2400 grams, was firm in consistence and had externally a mottled hemorrhagic appearance. When the liver was sectioned, the hemorrhages appeared irregular in outline and varied greatly in size, obscuring the lobulation.

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vessels of arteriolar size had greatly thickened, acellular wells.

In the liver were numerous focal hemorrhages, chiefly in the vicinity of periportal zones. These varied in size from a few extravasated red cells to masses which involved several contiguous lobules. Necrosis of liver cells accompanied the hemorrhagic lesions. Fragmented cells were seen in the interiors of leukocytes and large mononuclear cells. Large collections of lymphocytes were present around the periportal vessels, but none of the latter were thrombosed.

#### Case 3666

(For details of history see (1), Case 3.) In 1919 second pregnancy terminated at 8 months for toxemia. In July, 1921, miscarriage at 5 months, at which time there was distinct edema. In November, 1921, during third pregnancy, developed cold in chest with cough, followed a few days later by chills, dyspnea, orthopnea, headache, vomiting, pains in chest, limbs and back, and blurred vision. The condition became worse steadily until death 4 weeks after the onset of symptoms.

*Necropsy.* Both kidneys were small, the right weighing 55 grams and the left 31 grams. Their external surfaces were irregular and scarred. The pelves were greatly dilated; the cortex and pyramids were compressed to form a rim only 5 to 10 mm. in width. Cortical striations could be seen not at all or only with great difficulty. Both ureters were dilated, but the urinary bladder was small and contracted; its wall measured 12 mm. in thickness. The entire urinary tract was filled with thick, purulent material. The mucosa of the pelves, ureters and bladder was thick, opaque and contained numerous small hemorrhages.

The heart was increased in size, weighing 300 grams (body weight 38 kilos), and the papillary muscles were hypertrophied. There was no evidence of chronic passive congestion of the viscera and no edema nor ascites.

*Microscopic.* Extensive scars infiltrated with numerous lymphocytes and plasma cells replaced much of the renal parenchyma. Enormously dilated tubules with flattened epithelial cells and coagulated material in their lumina alternated with atrophic tubules. The majority of the glomeruli were replaced by densely hyalinized connective tissue; a few, less severely injured, had thickened capsules and an occasional adhesion between tuft and capsule. Both arteries and arterioles had concentrically thickened walls which reduced the size of their lumina.

The mucosa of the pelves, ureters and bladder was much thickened in some places. In others it was necrotic or desquamated and replaced by chronic granulation tissue containing numerous capillaries, lymphocytes and plasma cells which extended into the submucosa (Figure 4). A hemorrhagic exudate was seen on the free surface.

#### Case 79045

Born 1899. Patient is reported to have been entirely well during pregnancies in 1915, 1918, 1920, 1923, 1926 and 1927. All except the last, which ended in a pre-



mature delivery at 7 months, resulted in living children. Her mother died at 40 in childbirth, her father at 58 of "acute indigestion"; one sister at 44 had hypertension. She was first seen in the prenatal clinic March 21, 1929, three and a half months before term, with blood pressure 180/120, moderate edema of the ankles, heart slightly enlarged to the left, but no albuminuria. April 4, the blood pressure was only 130/80, but it was again 170/120 on April 22, still without albuminuria. April 29, she was admitted to the hospital in labor and delivered of a dead premature fetus. May 12, when she was discharged, the blood pressure was still 150/94. Her course in hospital was uncomplicated except that her temperature rose to 100.2° on May 4. She was again seen in the prenatal clinic April 16, 1931, in the sixth month of pregnancy. She had suffered from nocturia without diurnal frequency, urgency or burning, since the onset of pregnancy. April 9, she was seized with headache and began to vomit continuously. She had also been troubled with palpitation and with precordial pain which radiated to the back and down to the left hip. When seen, her blood pressure was 220/140, her heart was enlarged to the left and a soft diastolic murmur could be heard over the whole precordium, especially over the aortic area, and over the vessels of the neck. She also had moderate pitting edema of the legs. The optic discs were blurred and the retinal vessels tortuous. She was brought into the hospital, where she remained one week, at the end of which her blood pressure was still 156/110. Her course was afebrile. The urine contained the faintest possible trace of albumin; the blood nonprotein nitrogen was 18 mgm. per cent, phenolsulphonaphthalein excretion 80 per cent. She refused to permit termination of pregnancy and sterilization. June 29, she was readmitted to the hospital in labor and was delivered of twins precipitately. Her blood pressure was 250/150. July 2, she had an attack of intense precordial pain which persisted. July 3, she suddenly lost consciousness and stopped breathing. Although she roused for a short time, she died 30 minutes later. The urine on June 29 contained a slight trace of albumin and occasional granular casts. On June 30 the blood nonprotein nitrogen was 23 mgm. per cent; blood count, red blood cells 4.1 million, hemoglobin 70 per cent, leukocytes 5,500.

*Necropsy.* The kidneys, which weighed 175 grams each, were deeply congested and swollen, without evidence of scarring. The larger renal vessels were not thickened. The pelves and ureters were not dilated.

The liver weighed 1800 grams and did not appear abnormal in the gross. Microscopically there were small foci of necrosis in the periportal regions.

The heart was greatly hypertrophied, weighing 600 grams. Fresh blood clot filled the pericardial sac and unclotted blood oozed from a small opening on the anterior surface of the ascending aorta just below the pericardial attachment. This blood had its origin from a dissecting aneurysm which seemed to arise at the site of an intimal tear above one sinus of Valsalva on the posterior wall of the aorta and extended down as far as the superior mesen-

teric artery. There was only slight atheromatous change in any of the larger arteries, including the coronaries. Blood-tinged fluid was present in both pleural cavities, 200 cc. in the right and 500 cc. in the left.

The uterus projected about 10 cm. above the symphysis pubis, but was firm and contained only fresh blood clots in its cavity.

*Microscopic.* Although there was extensive cloudy swelling of the epithelial cells of the renal tubules, only occasional tubules were necrotic and in still fewer did the epithelium show colloid change. All the glomeruli had thickened basement membranes beneath the lining epithelium, similar to the glomerular changes in the first two cases. There was neither endothelial nor epithelial proliferation. A few glomeruli contained fibrin masses in their tufts, resembling the glomerular changes seen in malignant nephrosclerosis. The most constant lesion was a marked hypertrophic thickening of the media of the smaller renal arteries, which involved especially afferent glomerular arterioles, but affected also the arterioles generally throughout the body. The renal pelves were free from inflammatory reaction.

#### Case 83867

Born in 1898, patient was first seen June 25, 1930, early in pregnancy. She was reported to have had slight kidney trouble near the end of her first pregnancy in 1921, but this she denied. In 1925 she was, however, put to bed because of "inflammation of the kidneys," high blood pressure and edema of the ankles in the seventh month of her second pregnancy, which ended prematurely with a stillbirth. Otherwise she had no history of serious illness. There had been no symptoms of renal, cardiac or vascular disease, although she had been told that her blood pressure was slightly elevated. She appeared nervous and excited, pulse very rapid, blood pressure 192/120; the retinal vessels were normal; a systolic murmur was heard over the whole precordium. When she was admitted to the hospital for 6 days observation June 27 her pulse was 110, blood pressure 185/125; the urine contained no albumin and only an occasional leukocyte; the blood nonprotein nitrogen was 29 mgm. per cent; blood count normal. The blood pressure, taken by competent nurses and interns on several occasions varied from 132 to 140/70 to 95, although it was found on one occasion when she was excited, as high as 164/110. After discharge she remained entirely free from symptoms. Her blood pressure continued extremely variable. Although it was usually elevated, 150 to 200, when she reported to her physician, and showed a tendency to mount gradually, on occasions in her home it proved to be almost normal. The urine was consistently free from albumin. November 22, she noticed general malaise and myalgia and the next night was seized with sudden agonizing pain in the epigastrium and between the scapulae, accompanied by nausea and vomiting. The pain and vomiting recurred at intervals during the night. When she entered the hospital November 24 her pulse was 100, blood pressure 210/70;

her heart was enlarged to the left, with loud, blowing systolic murmurs in the aortic area and at the apex; there was slight dulness below the angle of the right scapula. November 27, her face was somewhat puffy, the murmurs at the apex and base grating in character; the liver was somewhat enlarged and râles were heard at the bases of both lungs. November 30, she appeared restless, apprehensive, pale, slightly cyanotic, with labored breathing, râles all over her chest, liver considerably enlarged, but no edema. December 2, the cyanosis and dyspnea had become extreme. The temperature, which had been as high as 100 to 100.4° occasionally, from the first, had reached 102.4° December 1. The heart sounds had become distant, the area of cardiac dulness had increased; the murmurs at the apex were fainter, but the to and fro hum at the base persisted. She suddenly stopped breathing early the next morning. The blood pressure varied from 140/70 to 200/80 quite capriciously. The urine contained much albumin, many leukocytes and casts and rare red blood cells. There was nothing unusual about the blood count except a leukocytosis 12,400 to 17,300 with 76 to 80 per cent polynuclears. November 26, the blood nonprotein nitrogen was 29 mgm. per cent, phenolsulphonaphthalein excretion 20 per cent, urine culture sterile. December 1, *Streptococcus viridans* was found in one blood culture; but 4 previous and 2 subsequent cultures proved sterile.

**Necropsy.** There were no abnormalities in either the fetus and its membranes or the uterus, which filled the pelvis and extended 18 cm. above the symphysis pubis.

The right kidney weighed 200 and the left 100 grams. This discrepancy in weight was due to several deep scars in the left kidney, the results of healed infarcts. The pelves and ureters were not dilated and contained no exudate. There were no evidences of marked atherosclerosis in the larger renal vessels and no scars in the cortex other than those already mentioned.

The liver weighed 1725 grams and externally and on section had a nutmeg appearance.

There was marked hypertrophy of the heart, which weighed 500 grams, and slight thickening of the mitral valve with shortening of its chordae tendineae. A large quantity of unclotted blood, filling the pericardial sac, was seen to emanate from a small discolored zone on the posterior surface of the ascending aorta. This vessel was the seat of a dissecting aneurysm which appeared to arise from a transverse break in the intima above the posterior cusp and extended down into the common iliac arteries and up into the left common carotid artery. There was no free fluid in the peritoneal cavity, but the pleural cavities contained 200 and 150 cc. of clear serous fluid.

**Microscopic.** There was no cellular inflammatory process in any part of the kidneys, including the pyramids and pelvis. The epithelium lining the convoluted tubules showed cloudy swelling of a degree sufficient to occlude the lumina frequently, but there was no epithelial necrosis or desquamation. All the glomeruli appeared to be increased in size, filling their respective

glomerular spaces; yet they were relatively acellular. Many of the capillary tufts had a wire-loop appearance, as the result of thick bands of homogeneously stained material between the rows of epithelial and endothelial cells. Some tufts appeared completely hidden by the deposition of an amorphous material like fibrin or coagulated fluid. The renal arterioles were all nearly occluded by a thickening of their media and the same vascular change was present in nearly all the other viscera. The tubular and glomerular lesions which were more acute in nature and similar to those found in the eclamptic cases, were superimposed on this older vascular disease.

Throughout the liver large zones of necrosis were seen, located principally in the periportal zones and for the most part unassociated with hemorrhage.

#### Case 81551

Born 1902. Past history unknown, except that her mandible was removed in 1910. About October 28, 1929, six weeks before term, in her first pregnancy, her ankles swelled. November 7, slight vaginal bleeding began. November 9, she was seized with sudden abdominal pain and vomited. Presumably she also had a convulsion. When she was brought to the hospital a little later she was restless, tossing, complaining of headache and abdominal pain, with blood pressure 210/140, marked pitting edema of the feet, ankles, legs and back, urine containing large amounts of albumin and casts, but no bacteria. She died in a *convulsion* before effective treatment could be instituted.

**Necropsy.** The gravid uterus filled most of the maternal pelvis and extended into the abdominal cavity to the level of the second lumbar vertebra. There were nearly 3 liters of slightly blood-tinged fluid in the peritoneal cavity.

The kidneys, which weighed 175 grams each, were not granular and their pelves and ureters were undilated.

The liver weighed 1800 grams and presented numerous large and small hemorrhages externally and on section. These were largest in the vicinity of the hepatic ligaments and gallbladder.

The heart was not hypertrophied, its weight being 300 grams (body weight 69 kilos).

**Microscopic.** There was no inflammatory reaction in the renal pelves and pyramids. Casts of desquamated epithelial cells were found in the collecting tubules. The epithelial cells of the convoluted tubules showed marked cloudy swelling. Some lacked distinct cell outlines and nuclei and had coarse granules in the cytoplasm. Most glomeruli appeared anemic. The tufts had sharp, unusually prominent outlines due to a thickening of the substance between capillary endothelium and lining epithelium. There was no epithelial or endothelial proliferation and no capillary occlusion. The renal arterioles did not have thickened walls.

In the liver the hemorrhagic zones were limited almost without exception to the periportal regions. Here the liver cells were crowded, flattened and necrotic.



occasionally the necrosis was found in small foci which were free from hemorrhage. Homogeneous, pink-staining material resembling hyalin or fibrin filled some of the periportal vessels.

#### *Case 61702*

Born 1893. Patient was admitted to the hospital September 10, 1927, 35 weeks gravid. Three previous pregnancies are reported to have been entirely normal. For a month she had suffered from frequent headaches, dizziness and slight edema of the feet. September 8, she developed photophobia and just before admission became suddenly blind. On admission she was restless and apprehensive, but rational, with marked tenderness over the frontal and maxillary sinuses, extreme retinal edema, blood pressure 170/85, moderate generalized subcutaneous edema, leukocytosis, urine containing much albumin, a few casts and leukocytes. A little later she had a severe convulsion, lasting 30 minutes, but after this she improved so that she was able to take fluids the next day. However, her temperature had risen to 101°. September 12, her blood pressure fell to 130/40 and the temperature became normal. At 8:00 p.m. the fetal heart suddenly ceased to beat. A Voorhees bag was inserted and 4 hours later she was delivered of a premature child which lived less than 24 hours. September 15, the temperature rose to 100° F., the pulse was about 100, blood pressure 140/100. Her general condition became progressively worse. September 20, she sank into coma; September 21, she exhibited signs of cerebral irritation and focal pneumonia; September 22, she died with a terminal rise of temperature to 106°. The Van den Bergh test, September 10, was entirely negative. Spinal fluid obtained after death, by puncture of the cisterna, was xanthochromic and contained many red blood cells.

*Necropsy.* Small, fresh hemorrhages were seen on the serosal surfaces of the ovaries and uterus. There were no thrombi in the vessels of the broad ligaments. The uterus was soft and enlarged and on section revealed a large blood clot which was adherent to the anterior surface of the endometrium.

In the cortex of the kidneys, which weighed 175 grams each, there were minute fresh hemorrhages. The right kidney also contained a small white infarct. The renal pelvis and ureters were not dilated and were free from purulent material.

The liver weighed 1800 grams and presented a normal appearance in the gross and on microscopic examination.

Numerous large and small hemorrhages were seen in both lungs. Leading to a few of these which were raised above the pleural surfaces, could be traced blood vessels which were occluded by friable blood clots. In a few instances these hemorrhagic infarcts extended to the pleural surface which, however, was still free from exudate and fluid. The right adrenal was hemorrhagic and twice the size of the left which weighed 25 grams. Recent small hemorrhages were scattered in the white matter of both cerebral hemispheres and in the left thalamic nucleus.

*Microscopic.* Several of the larger uterine veins were

occluded by thrombi which in some instances were in process of organization. These and other vessels were surrounded by small mononuclear cells resembling lymphocytes and by small numbers of leukocytes. The source of the emboli to the lungs, brain, right kidney and adrenal appeared to be these uterine thrombi. The infarcts in these organs were still quite recent, with little evidence of organization and none of suppuration.

The renal pelvis were devoid of inflammatory exudate. In the tubules, especially their convoluted portions, was noted extensive injury consisting of marked swelling of the epithelium which often led to tubular occlusion. Numerous necrotic epithelial cells were desquamated into the lumina. All the glomeruli showed some thickening of the basement membrane and a relative acellularity. None of the capillary loops were occluded. Small hemorrhages were present in the interstitium of the cortex. The renal vessels, large and small, were not altered in any way.

#### *Case 9354*

Born 1898. July 24, 1922, two months before term, in her first pregnancy, patient had a normal blood pressure and no albuminuria. Shortly after this she developed edema of the feet which gradually extended to the legs; and about August 5 extreme nocturnal urinary frequency. August 12, she awoke with a headache which increased steadily during the day. At 4:00 p.m. she had a convulsion, followed by 3 more in rapid succession. At 7:00 p.m. when she entered the hospital she was conscious, but restless and breathless. She had generalized edema, a totally irregular heart, blood pressure 142/115. At 8:00 p.m. the blood pressure was 175. Convulsions continued at intervals. At 10:00 p.m. the fetal heart became inaudible. At 3:00 a.m., August 13, she lapsed into coma after a convulsion. At 4:00 a.m. she was delivered of a dead child, a little later the blood pressure fell sharply to 90/55, and at 7:00 a.m., after her 12th convulsion, she died. Urine obtained by catheter on admission contained much albumin, many casts, and masses of red blood cells and leukocytes. She did not urinate again, and only a few drops could be obtained by catheter after death. The temperature rose to 101° a few hours before death. Blood count: red blood cells 5.1 million, hemoglobin 85 per cent, leukocytes 30,800, polynuclears 85 per cent. Blood nonprotein nitrogen 28 mgm. per cent.

*Necropsy.* A subserous myoma measuring 5 cm. in diameter was found on the posterior wall of the greatly enlarged uterus. The uterine cavity was filled with a fresh blood clot, but otherwise was not unusual.

The left kidney weighed 175 and the right 150 grams. Both showed the remains of fetal lobulations and were pale and swollen. The pelvis and ureters were not dilated.

The liver weighed 1875 grams. Parts of it were soft and flabby, yellow in color and indistinctly lobulated.

A recent hemorrhage some 2 cm. in diameter was present in the lower lobe of the right lung.

*Microscopic.* There was a moderate amount of granulation and irregularity of the epithelium of the convoluted and collecting tubules of the kidneys. Some tubules were obstructed by the swollen epithelium, some were filled with granular material and some with numerous well preserved red blood cells. The glomeruli were not abnormal. There was no pyelitic process and the renal vessels were not thickened.

There were numerous zones in the liver in which the hepatic cells were poorly stained. Some of the cells had granular cytoplasm and small, pyknotic nuclei. There was no definite necrosis and no hemorrhage.

A few cortical extravasations of red blood cells were found in the adrenals.

#### Case 60242

Born 1905. Patient was reported to have been treated for kidney trouble for about a year in 1923, but the nature of the disease is not known. April 8, 1927, 12 days after the birth of her first child, although labor and delivery had apparently been uncomplicated, she was seized with sudden cramp-like pain in the lower abdomen, followed by a chill. The pain lasted only a short time. On the morning of April 9 she had a severe headache, but took some food. Later in the day she vomited several times, the last times bloody material. Meanwhile she became increasingly languid, finally lapsing into stupor with incontinence of urine. She was brought to the hospital in a comatose state, pale, with slight puffiness of the face, rapid, shallow breathing, pulse 140, blood pressure 80/50, with no demonstrable lesions in heart, lungs or abdomen. She did not respond to infusions, transfusions and other stimulative measures. April 10, she appeared slightly jaundiced. Later in the day she developed strabismus and ptosis. She continued to vomit blood-stained material. September 11, she appeared extremely jaundiced, cyanotic, restless, with gasping respirations, pulse 160, blood pressure 64/52, tenderness over the whole right side of the abdomen. At noon she suddenly vomited blackish material and died a few minutes later after some convulsive movements. Urine, obtained by catheter, contained much albumin and moderate numbers of leukocytes. Blood count: red blood cells 4.9 million, hemoglobin 85 per cent, leukocytes 22,200, polynuclears 94 per cent. Blood culture negative. Spinal fluid (postmortem) clear, globulin  $\pm$ , leukocytes 121, mononuclear 118, polynuclear 3, red blood cells 152, culture sterile, Wassermann negative. Blood nonprotein nitrogen, April 11, 98 mgm. per cent. The temperature, on admission, 105°, was continuously elevated until death.

*Necropsy.* Although it was still increased in size and soft, no further abnormalities of the uterus were discovered either in the gross or on microscopic examination. This was true as well of the tubes and broad ligaments.

The right kidney weighed 150 and the left 125 grams. Both were unusually pale and appeared somewhat swollen. Their pelves and ureters were not abnormal.

The liver weighed 1425 grams and appeared normal

macroscopically. There were likewise no lesions in the brain.

*Microscopic.* There was no cellular reaction in the renal pelves and pyramids. Extensive edema was present in the connective tissue throughout the cortex and medulla. The renal tubules contained a flattened epithelium even in the convoluted portions, but the cytoplasm was markedly granular. The nuclei were often absent or obscured and many cells were desquamated. This extensive tubular lesion overshadowed the milder glomerular changes which consisted of an increase in their size without an apparent increase in their cellularity and of a slight thickening of the connective tissue between capillary endothelium and lining epithelium. The renal vessels were not thickened.

Throughout the liver were seen numerous foci of necrosis and hemorrhage (Figure 5). These were invariably of small size and almost invariably located in periportal regions. Small numbers of leukocytes were found among the necrotic liver cells. The periportal vessels did not appear occluded.

#### Case A32601

Born 1896. Patient is reported to have had "no serious illnesses nor operations." Previous pregnancies in 1925, 1928 and 1931, the last ending in abortion, are said to have been uncomplicated. In October 1934, about 3 months before term, her feet began to swell and after this she suffered from slight headaches. When seen, December 19, she had edema of the abdominal wall and legs, blood pressure 160/110. The next morning at 5:00 a.m. her pulse had risen to 120 and she had a slight cough. By 8:00 a.m. her sputum was bloody, pulse 144, breathing rapid, skin clammy, râles were audible at the left base and in the right scapular region. Dyspnea and cyanosis increased steadily. On the morning of December 21, râles became audible over the whole chest, the pulse became weak and irregular, and the blood pressure fell to 90/60. She died a few hours later. The temperature remained normal until a few hours before death, when it rose to 102°. The urine contained much albumin and moderate numbers of red blood cells and leukocytes. Blood count: red blood cells 3.9 million, hemoglobin 60 per cent, leukocytes 16,500, polynuclears 92 per cent.

*Necropsy.* The gravid uterus contained a twin pregnancy, but was otherwise not unusual.

The right kidney weighed 216 and the left 182 grams. Both were pale, flabby, and considerably swollen. Their pelves were not dilated, but the right ureter was approximately 3 times the diameter of the left. This dilatation extended from the brim of the maternal pelvis to the renal pelvis. The wall of this ureter was also considerably thickened.

The liver weighed 1985 grams and on section revealed a sprinkling of small, bright red hemorrhages.

Both lungs were extensively consolidated with pneumonia and were deep red in color.

*Microscopic.* Although there was widespread cloudy swelling of the renal tubular epithelium, few of the

cells were desquamated. Nevertheless, the lumina were filled with an amorphous granular material and occasional red blood cells. The glomerular tufts were unusually prominent by virtue of thickened subepithelial connective tissue, which produced the "hair-pin" appearance often described in eclampsia. The glomeruli were large and filled their respective spaces without any apparent increase in their cellularity. The renal blood vessels did not have thickened walls.

Numerous foci of cellular necrosis were found in the liver. Some were quite small while others involved portions of several adjacent liver lobules. They occurred relatively infrequently in the periportal zones and had no special site of predilection within the lobules. Only infrequently were they hemorrhagic, but almost all contained leukocytes.

#### *Case 71013*

Born 1895, colored, patient was admitted to the hospital August 7, 1928. Four months earlier pregnancy was terminated because of orthopnea, dyspnea and edema of the feet. The symptoms, however, had increased steadily after this. On admission she was acutely sick, confused and semistuporous, with orthopnea, dyspnea and massive edema of the lower part of the trunk and the lower extremities. The left tonsil was much enlarged, the posterior cervical lymph nodes palpable. There was some fluid in the left chest, more in the right, with râles audible above the fluid. The heart was enlarged with a harsh systolic murmur and a rough diastolic murmur at the apex, the liver was enlarged almost to the navel. She seemed to improve under treatment for a few days; but it was impossible to restore cardiac and renal function and to eliminate edema. August 26, she sank into a stupor and died. The blood pressure, August 25, was 230/160. She had a slight irregular fever. The Wasserman reaction was 4+ cholesterol, 0 alcoholic antigen. The urine, of low specific gravity throughout, contained much albumin, many leukocytes and casts. Blood count: red blood cells from 4.4 to 1.6 million, hemoglobin from 60 to 30 per cent; leukocytes were only 8,000 on August 9 and 17, but 20,200 on August 25. Blood nonprotein nitrogen: August 8, 89 mgm. per cent, August 23, 187 mgm. per cent. Phenolsulphonephthalein excretion, August 21, 2 per cent. Cultures of fluid from thorax and abdomen were sterile.

*Necropsy.* The peritoneal cavity contained about 1500 cc. of clear serous fluid in which were floating flakes of fibrin, and each of the pleural cavities contained about 500 cc. of similar fluid.

Both kidneys, which weighed 100 grams each, were edematous and pale and had numerous cortical scars. The pelvic mucosa was pale and intact, but appeared somewhat thickened. The pelves and ureters were not dilated.

The external surfaces of the liver were covered with strands of fibrin which were present also in the peritoneal cavity. The organ weighed 1400 grams and was mottled yellow and red in the pattern of a "nutmeg" liver.

Small hemorrhages were scattered beneath Glisson's capsule.

The hypertrophied heart weighed 500 grams (body weight 75 kilos). There were no valvular lesions to account for this hypertrophy and no obvious arteriosclerotic lesions were found in any of the organs.

*Microscopic.* The renal pelves were lined by a hyperplastic mucosa like that seen in chronic pyelitis, but there were few inflammatory cells in or beneath the mucosa. Extensive scars infiltrated with small mononuclear cells marred the renal architecture (Figure 6). These scars undoubtedly accounted for the numerous shrunken, atrophic renal tubules which occurred in groups adjacent to greatly dilated and hypertrophied tubules. Surprisingly large numbers of tubules were filled with granular and cellular casts in which polymorphonuclear leukocytes predominated. The glomeruli were much less extensively injured. Many were hypertrophied and small numbers were completely hyalinized. In rare instances adhesions were seen between glomerular tufts and Bowman's capsule. There was simple hypertrophy of the media of the renal arteries. The smaller arterioles had such greatly thickened walls that the lumina were frequently obliterated.

The liver lobules showed extensive necrosis and hemorrhage in the central vein regions. With this picture of chronic passive congestion there was no demonstrable fibrosis.

#### *Case 18925*

Born 1900. Patient was admitted to the hospital in labor in the 8th month of pregnancy, June 14, 1923. She is reported to have had edema for 2 months in 1920, with recurrences in the summers of 1921 and 1922. For a month before admission she had moderate, generalized edema; for 2 weeks headaches, dizziness and blurred vision. On admission she presented moderate, generalized subcutaneous edema, blood pressure 180/136. Because she was bleeding from the uterus a Voorhees bag was inserted. A little later she was seized with severe epigastric pain, vomited, and went into a state resembling shock, with air-hunger, although her blood pressure fell only to 150/118. She improved after a transfusion, but her temperature rose to 100° during the day and on succeeding days rose still higher, varying from 100 to 104°. June 15, she became cyanotic. The edema increased, respirations became labored; the abdomen was distended, everywhere tender, with dulness in the flanks, and signs of fluid appeared in the right chest. She was almost anuric, and the edema increased steadily. She sank into coma and died June 20. The urine contained much albumin, casts, pus cells and red blood cells. Blood count: red blood cells 2.9 to 2.1 million, leukocytes 30,600 to 25,200, polynuclears 89 to 93 per cent. Blood nonprotein nitrogen June 14, 51 mgm. per cent; June 15, 99; June 18, 181; June 20, 221.

*Necropsy.* The uterus was enlarged, rising slightly above the brim of the maternal pelvis. The Fallopian tubes were deeply congested. There were 150 cc. of

cloudy fluid in the peritoneal cavity whose surfaces, however, were smooth and glistening.

The kidneys weighed 200 grams each. They were pale organs from which the capsules could be stripped readily, exposing smooth surfaces covered with great numbers of petechiae. Neither the pelves nor ureters were dilated.

The liver was very large, weighing 2200 grams. It contained numerous large central zones of necrosis which were yellow in color surrounded by peripheral zones of hemorrhage. Similar lesions were found in the lungs and colon.

Two large, cauliflower-like masses were attached to the mitral valve of the heart which weighed 275 grams. One of these was quite firm and gray in color, the other was red and friable.

*Microscopic.* The endometrial surface of the uterus was covered by blood clot which was infiltrated with leukocytes and contained great masses of bacteria. Extensive hemorrhages were present throughout the uterine musculature. Many vessels were filled with thrombi, some of which were partially organized.

There was extensive destruction of the tubular epithelium of the kidneys. Many tubules were completely filled with granular material which was often mixed with mononuclear cells and leukocytes. Some contained casts of red blood cells. The interstitial tissue was edematous and contained both cellular exudate and bacterial masses. All the glomeruli were swollen and filled their spaces completely. Some were hemorrhagic and others contained bacterial emboli in their capillary loops. Many of the renal vessels were congested, but they were not otherwise altered. The pelvic mucosa appeared normal.

Bacterial emboli in large numbers were seen in the liver, lungs and colon. Microscopically, infected infarcts were found in all these organs.

#### Case 33564

Born 1905. Patient was admitted to the hospital June 13, 1924, about the sixth month of her first pregnancy. For two months she had been extremely irritable and nervous, with increasing awkwardness in manual movements and twitching of the facial muscles. June 6, she suddenly developed extreme weakness of the legs, and a little later grimacing and difficulty in speaking. June 10, she was forced to bed by weakness of the legs, pains in the pelvis, abdomen, loins and hips. June 13, she gradually sank into coma. On admission she was in deep coma, moaning, restless and twitching, temperature 103.4° pulse 160, blood pressure 120/86, with rapid, labored breathing, tracheal râles, slight cyanosis, dry coated tongue, rigid neck and spine, apical thrill and murmur, doubtful Kernig sign and absence of deep reflexes. Her condition did not improve. The temperature rose steadily to reach 107° before death on June 15. Urine contained much albumin, many casts, and occasional red blood cells and leukocytes. Blood count: red blood cells 5.2 million, leukocytes 12,400, polynuclears

83 per cent. Blood culture sterile. Spinal fluid clear, but under increased pressure. Blood nonprotein nitrogen 29 mgm. per cent.

*Necropsy.* The gravid uterus filled the maternal pelvis and extended up to the level of the umbilicus. The fetus and fetal membranes presented no abnormalities.

The kidneys, which weighed 200 grams each, were swollen and congested. A few petechiae were seen in the mucosa of the renal pelves. The latter as well as the ureters were not dilated.

A row of verrucous vegetations were present along the line of closure of the mitral valve. The heart, which weighed 225 grams, presented no other abnormalities.

Lack of permission prevented examination of the brain.

*Microscopic.* The renal tubular epithelium was swollen and granular and in some places had undergone actual necrosis. The tubules contained amorphous granular debris, desquamated cells and erythrocytes. The interstitial tissue was edematous and here and there contained collections of small round cells. There was marked congestion of the glomeruli whose capillary endothelium was swollen. Some glomerular capillaries appeared closed with thrombi. Other than congestion the renal vessels presented no abnormalities. The pelvic mucosa was normal.

Typical Aschoff bodies were found in small numbers throughout the myocardium. The mitral verrucae consisted of hyalin thrombi superimposed on an organizing exudate composed of round cells and leukocytes. Bacteria were not present.

The liver was normal histologically.

#### Case 63494

(For details of history see (1), Case 4.) In 1928, just after delivery of first child went into *eclampsia*. Puerperium febrile, associated with pyuria. Relapse, after discharge, with fever, headache, lumbar pain and general malaise. Ten weeks after delivery, hypertension. Readmitted 5 years later with advanced renal and cardiac failure and died after 5 weeks.

*Necropsy.* The right kidney weighed 70 and the left 60 grams. The cortical markings were indistinct and much of the parenchyma seemed to have been replaced by fibrous connective tissue. The surfaces of the kidneys were markedly granular. Both kidneys contained a superabundance of peripelvic fat although the pelves were not dilated. Nor were the ureters unusual except for their greatly thickened walls.

There was moderate cardiac hypertrophy, the heart weighing 450 grams (body weight 74 kilos). There were no valvular nor vascular lesions. An excess of clear, yellow, serous fluid (175 cc.) was found in the pericardial sac. There were 125 cc. of similar fluid in the left pleural cavity; the right cavity was obliterated by fibrous adhesions.

*Microscopic.* The architecture of the kidney was distorted by a tremendous increase of connective tissue. In many zones the glomeruli were completely hyalinized and their associated tubules were atrophic. Some glo-

meruli were greatly hypertrophied and their tubules were dilated and lined by tall columnar epithelium. The cytoplasm of some of the cells lining these tubules showed colloid change. There was an extensive lymphocytic infiltration throughout the interstitial tissue. The blood vessel walls were thickened, but their lumina were patent. The picture presented by both kidneys was that of advanced hydronephrosis. The pelvic mucosa was desquamated in many places and its place was occupied by lymphocytes, plasma cells and large mononuclears (Figure 7). The walls of both ureters were greatly thickened as the result of an increase of fibrous tissue. Beneath and replacing the ureteral mucosa were collections of lymphocytes, other mononuclear cells and plasma cells (Figure 8).

The liver was normal.

#### Case 44154

(For details of history see (1), Case 5.) In 1912 pyelitis in seventh month of first pregnancy. After this, complaint of recurrent pains in back and abdomen with later hypertension, renal and cardiac failure until death in 1935.

*Necropsy.* There were generalized subcutaneous edema, 800 cc. of clear, amber fluid in the peritoneal cavity and 300 cc. of similar fluid in the right pleural cavity. The left thoracic cavity was obliterated by fibrous adhesions.

The right kidney weighed 24 grams and the left 60 grams. The external surfaces of both were scarred and granular. The right kidney consisted largely of its pelvis and calices with only a narrow rim of cortical tissue. On the left side there was no obvious pelvic dilatation. Both ureters were undilated. Large subintimal atheromatous plaques were present in both renal arteries, but they were more numerous in the right.

The liver weighed 1230 grams and showed numerous small hemorrhages externally and on section.

There was cardiac hypertrophy, especially of the left ventricle, without cardiac dilatation, the heart weighing 395 grams (body weight 50 kilos).

The wall of the entire colon was thickened by edema and its mucosa was discolored and ulcerated in numerous places. The ulcerations surrounded small islands of preserved mucosa which resembled polypoid structures.

*Microscopic.* Both kidneys were tremendously scarred and contained little normal parenchyma. The scars were infiltrated with large collections of lymphocytes and other mononuclear cells. The tubules were either shrunk and filled with coagulated homogeneous material or were greatly dilated and lined by hypertrophied epithelium. The majority of the glomeruli were completely hyalinized. Both large and small arteries had markedly thickened walls with hyperplastic intimal coats and minute lumina. Several vessels were filled with a loose reticulum of connective tissue. In the pelvic regions the kidneys were composed of dense fibrous tissue which was invaded by small numbers of lymphocytes.

Occasional hepatic lobules revealed both necrosis and

hemorrhage in the region of the central veins. In some, there was also central fibrosis.

A hemorrhagic fibrinopurulent exudate replaced the mucosa and infiltrated the submucosa of the colon. Here as in most of the other organs of the body the smaller arteries showed concentric intimal thickening.

#### Case 53431

(For details of history see (1), Case 11.) Pyelitis and toxemia in 4th pregnancy in 1929. In 1934 died of pulmonary embolism following appendectomy and uterine suspension. Before this some urinary symptoms and inconstant hypertension.

*Necropsy.* A dense, red, friable blood clot 10 cm. in length occluded the right pulmonary artery. Most of this lung was hemorrhagic from early infarction. The source of the pulmonary embolus could not be determined.

The kidneys, which weighed 150 grams each, had smooth external surfaces, and were deeply congested. The mucosa of both renal pelves was thickened and had a milky appearance, but only the right pelvis and ureter were dilated.

The heart was not hypertrophied, weighing but 250 grams, and the liver was not abnormal.

*Microscopic.* There was extensive cloudy swelling of the renal epithelium which, however, was not necrotic. A marked vascular congestion involved both the pyramids and the glomeruli which were very large and filled their respective spaces completely. Only an occasional glomerulus was hyalinized, and there were relatively few cortical scars. The renal vessels were thickened but slightly. Most of the pelvic mucosa was desquamated; the submucosa was thickened with dense fibrous tissue free from inflammatory reaction.

#### Case 31841

(For details of history see (1), Case 8.) Pyelitis in 1918, aggravated by pregnancies in 1924 and 1926. After this, increasing urinary symptoms, hypertension, and renal and cardiac failure, ending fatally in 1932.

*Necropsy.* About 2 liters of clear, pale brown fluid were present in the peritoneal cavity, 1500 cc. in the right and 750 cc. in the left pleural cavity. The subcutaneous tissues throughout the body were water-logged. Numerous delicate fibrinous adhesions bound together epicardium and pericardium.

The right kidney weighed 50 and the left 60 grams. Both were scarred and granular externally and revealed extensive cortical scars on section. The pelves were not greatly dilated, but both were lined by thickened mucosa. The calices of the right kidney were filled with friable, black, calcareous material. The ureters were not dilated but their walls were greatly thickened. None of the larger renal vessels showed much atheromatous change. A small quantity of purulent urine filled the bladder whose mucosa was ulcerated over small areas and contained many petechial hemorrhages.

The heart was hypertrophied, weighing 425 grams

(body weight 68 kilos), but was not dilated. There was no chronic passive congestion in the liver.

*Microscopic.* There was widespread scarring with mononuclear cellular infiltration throughout both kidneys. Many tubules were atrophied and others were greatly increased in size. Cellular casts in which polymorphonuclear leukocytes predominated filled many of them. The majority of the glomeruli were strikingly altered. Some were entirely replaced by hyalinized connective tissue; in others there were adhesions between tufts and capsules. Still others, and these were perhaps in the majority, revealed amorphous material like fibrin in isolated capillary loops, presenting a picture similar to that seen in so-called malignant nephrosclerosis. The afferent arterioles of such glomeruli were often greatly thickened (Figure 9) and small numbers of leukocytes were present in the glomerular spaces. Nearly all the renal arterioles had markedly hypertrophied walls, but this vascular change was seen also in the pancreas, stomach and spleen. The mucosa of the renal pelvis revealed a marked round cell infiltration and a great increase of the underlying connective tissue (Figure 10). The ureters showed changes which were quite comparable to those in the pelvis. In the urinary bladder the mucosa was desquamated over regions which were infiltrated with small round cells. A few fresh hemorrhages also lay scattered in the submucosa.

#### Case 8250

(For details of history see (1), Case 7.) Kidney trouble after first pregnancy in 1910, succeeded by recurrences and exacerbations with pregnancies in 1914, 1918, 1920, 1922 and 1924. In pregnancy in 1926 extreme hypertension, urinary frequency and burning. Persistent hypertension, albuminuria and pyuria early in 1927, death a few months later from barbitol poisoning.

*Necropsy.* Both kidneys were small and diffusely scarred, the right weighing 57 and the left 35 grams. The capsules were thick, tough and firmly adherent to the underlying parenchyma. Each kidney had two dilated pelvis and two ureters. On the right side the ureters entered the urinary bladder by two separate orifices, on the left the two ureters fused near the bladder and entered it through a single orifice. All the ureters were dilated.

*Microscopic.* There was a diffuse increase of connective tissue throughout both kidneys. In these scars were collected many clumps of lymphocytes and other mononuclear cells. Large numbers of renal tubules were atrophic and filled with homogeneous coagulated material. Others were dilated and lined with tall columnar cells. Most of the glomeruli were completely fibrotic, but some were increased in size and filled their spaces. Occasionally glomeruli were found which had adhesions between tufts and Bowman's capsules. The large and small renal vessels had concentrically thickened walls and greatly narrowed lumina. The renal pelvis showed great congestion and edema with desquamation of the lining epithelium and granulation tissue formation

along the surface. The ureters displayed the same type of inflammatory reaction.

#### Case 47162

(For details of history see (1), Case 9.) In 1919, at 18, scarlet fever without nephritis, but slight albuminuria and hyposthenuria. Toxemia with pregnancy in 1920. After this, increasing cardiac and renal failure with hypertension until death in 1933.

*Necropsy.* Together the kidneys weighed 100 grams but the right was much smaller than the left and weighed approximately 15 grams. Both were greatly scarred, the renal capsules being firmly attached to the parenchyma. The right renal artery was quite small and hypoplastic but relatively free from atheroma. The renal pelvis and ureters of both kidneys were dilated to 2 or 3 times their normal size. The urinary bladder was likewise dilated and its wall was surprisingly thick. The urethra was narrow and although its lumen was patent, its wall was increased in thickness. The ureteral orifices were not obstructed.

The heart was not appreciably hypertrophied, weighing but 300 grams in a patient of 61 kilos. There was no evidence of chronic passive congestion in any of the viscera.

*Microscopic.* No glomeruli were present anywhere in the right kidney, which was replaced almost entirely by scar tissue in which were found dilated and atrophic tubules as well as numerous lymphocytes, large mononuclear cells and leukocytes. In the left kidney the findings were essentially similar but less intense. Many glomeruli were completely and some were partially hyalinized. There were striking vascular changes in both kidneys. These consisted of marked thickening of the walls and narrowing of the lumina of the arteries. The epithelium lining the renal pelvis was in part desquamated. There were subepithelial edema, vascular congestion and leukocytic infiltration. Leukocytes were frequently found in clumps within the collecting renal tubules. The ureteral walls were thickened and infiltrated with lymphocytes. The wall of the urinary bladder was thickened; its epithelium was desquamated and the lamina propria was infiltrated with polymorphonuclear leukocytes and contained congested blood vessels. There was an increase of connective tissue in the wall of the urethra.

#### Case A9526

(For details of history see (1), Case 6.) Pyelitis in first pregnancy in 1913. Three later pregnancies reported uncomplicated. After 1930 increasing symptoms of hypertension, renal and cardiac failure. Death in 1932.

*Necropsy.* There were 800 cc. of turbid, blood tinged fluid in the peritoneal cavity whose surfaces were covered with a thin film of fibrinopurulent exudate. The other serous cavities of the body were not abnormal.

Both kidneys were greatly diminished in size, the right weighing 70 and the left 80 grams. Their external surfaces were scarred and their cortical architecture was



blurred by reason of diffuse scarring. The pelves, calices and ureters were dilated to a moderate degree and were lined by a thickened membrane.

The heart was moderately hypertrophied, weighing 360 grams, while the body weight was 54 kilos. The only other abnormalities noted macroscopically were small fresh hemorrhages in the lungs, liver and mucosa of the gastro-intestinal tract and liver.

*Microscopic.* Much of the architecture of the renal parenchyma was replaced by scars which were diffusely infiltrated with lymphocytes, plasma cells and moderate numbers of leukocytes. Many collecting tubules were filled with casts which were composed of granular amorphous material, cast-off epithelial cells, or polymorphonuclear leukocytes. The convoluted tubules less often contained such casts. The changes most frequently encountered in the glomeruli were fibrosis and hyalinization. Usually the whole glomerulus was thus involved. Glomerular adhesions were infrequent, nor were there conspicuous changes in Bowman's capsule. Nearly all the renal vessels had markedly narrow lumina. The narrowing was in each instance due to medial hypertrophy and increase in subintimal connective tissue. There were capillary congestion and hemorrhagic extravasations in the subepithelial layer of the renal pelves and urinary bladder. An infiltration of lymphocytes, plasma cells and other mononuclear cells was also present beneath the pelvic and cystic mucosa.

#### Case 20067

Born 1896. Patient was first seen in the dispensary October 29, 1918, two weeks before term in her fourth pregnancy. Earlier pregnancies in 1912, 1913 and 1915 were reported to have been normal. The blood pressure was 110/85 and on December 4, 1917, the urine free from albumin on both occasions. She was delivered at home, without complications on December 24. June 2, 1921, she was seen again with incomplete abortion, but no general examination was made and she went elsewhere for treatment. In 1922 she had an abortion at 3 months for which she was treated by curettage in another hospital. In June or July 1923 another pregnancy was terminated early by self-induced abortion. November 19, 1923, she appeared in the dispensary again 3 months pregnant, complaining of pain in both lower quadrants of the abdomen since the onset of pregnancy. January 2, she complained of coryza, hoarseness and palpitation; her blood pressure was 108/62, urine clear. She continued to complain of pains in the abdomen and back, but was free from objective signs until May 15, when her blood pressure was 152/90. May 26 and June 2, her ankles were swollen, but blood pressure was normal. She was delivered at home June 4 apparently without complications. November 18, 1927, during another pregnancy, a diagnosis of cystitis was made, because of complaints of urinary frequency and pain and tenderness in the left lower quadrant of the abdomen, although her urine was clear and her blood pressure normal. She was delivered at home April 22 uneventfully, the urine and

blood pressure remaining normal throughout. June 4, the blood pressure was 112/80. April 17, 1931, complaining of sharp pain in the right side of her neck and the right shoulder, her blood pressure was 185/117, her urine contained a faint trace of albumin, the blood count was normal. Repeated examinations revealed nothing further. April 19, 1933, two months pregnant, complaining of pain in the right lower quadrant of the abdomen, palpitation, dyspnea and increasing urinary frequency, her blood pressure was 250/150, heart slightly enlarged with a systolic murmur at the apex, the optic discs blurred, tortuous vessels, scars and hemorrhages in the retinae, tenderness in both lower quadrants of the abdomen, urine containing a faint trace of albumin, rare leukocytes and casts, blood nonprotein nitrogen 26, blood count normal. She was admitted to the hospital where pregnancy was terminated by hysterotomy and the Fallopian tubes were sectioned and ligated. Symptoms and hypertension continued. March 16, 1934, her vision was failing, blood pressure 230/120, blood nonprotein nitrogen 36, phenol-sulphonaphthalein excretion 40 per cent. August 10, she was seized with sharp precordial pain, followed by dyspnea and palpitation. She entered the hospital acutely ill, apprehensive, with extreme dyspnea and orthopnea, obliteration of the optic discs and retinal hemorrhages, enlarged heart, coarse râles at the bases of both lungs, bilateral pleural effusion, but no subcutaneous edema. The urine contained much albumin, a few red blood cells and granular casts and moderate numbers of leukocytes; there was moderate secondary anemia, and the blood nonprotein nitrogen was 84 mgm. per cent. She did not respond to treatment and died August 22, with a terminal blood nonprotein nitrogen of 172 mgm. per cent.

*Necropsy.* About 400 cc. of clear, yellow fluid were present in each pleural cavity, but the other serous cavities did not contain an excess of fluid.

The right kidney weighed 140 and the left 145 grams. Their external surfaces were coarsely granular and their cortices were quite narrow. Both externally and on section there could be seen small fresh hemorrhages in the cortex. The renal pelves and ureters were not dilated or otherwise altered. The larger renal vessels did not appear to be thickened.

The heart was distinctly hypertrophied and weighed 495 grams (body weight 62 kilos). Its ventricles were dilated and there was evidence of chronic passive congestion in the liver and lungs.

*Microscopic.* In both renal cortex and medulla were numerous small scars infiltrated with small numbers of lymphocytes. The convoluted tubules showed cloudy swelling of their epithelium and often contained coagulated material in their lumina. The glomeruli presented a great variety of changes consisting of partial and complete fibrosis and hyalinization, adhesions between tufts and capsules, vascular congestion, and hemorrhage into glomerular spaces. In rare instances the epithelium of Bowman's capsule had proliferated to form crescents. A striking glomerular change was the thickening of the subendothelial connective tissue and the

presence of a fibrin-like material in the capillary tufts. The afferent glomerular arterioles were strikingly thickened with slit-like lumina and walls which seemed to be hyalinized. Similar changes were noted in the larger arterioles. The large renal arteries were relatively normal. There was no sign of an inflammatory reaction in the renal pelves.

#### Case 43495

Born 1898. Patient was first seen in the prenatal clinic, May 27, 1926, three months pregnant, with blood pressure 150/90. Pregnancies in 1921 and 1922 were reported uncomplicated, but in the third, in 1924, her blood pressure is said to have been high. On all subsequent visits until the end of pregnancy blood pressure and urine were found normal, and she was delivered in the hospital January 13, 1927, without difficulty. Blood pressures in May, 1927, were normal, March 11, 1929, she again came to the prenatal clinic, 4 months pregnant. August 26, the blood pressure which had been consistently normal, was 142/82, the urine loaded with albumin. August 28, she was admitted at term, blood pressure 156/100, eyegrounds normal, albuminuria profuse, no edema, blood nonprotein nitrogen 24 mgm. per cent. She was delivered August 30 and had an uneventful puerperium. October 7, in the dispensary, her blood pressure was 160/108; she complained only of pain and tenderness in the sacro-iliac region. Subsequent blood pressures: October 15, 130/80; November 19, 152/100; December 16, 130/100. The urine was normal February 4 and 7, 1930. March 17, she entered the hospital for dilatation and curettage, following an abortion in the third month, with blood pressure 150/90. November 6, it was 165/100, and September 18, 1931, 150/90. On this last occasion she complained of headache and blurred vision. May 28, 1932, when she entered the hospital for a perineal repair the blood pressure was 160/108, the heart slightly enlarged, urine containing moderate amounts of albumin and occasional leukocytes. Subsequent blood pressures, after operation, were 130/80, 135/90 and 132/92. June 24, in the dispensary, the blood pressure was 160/110, the urine clear. Early in September she began to suffer increasingly from headaches. After breakfast on September 17, she vomited, a little later she developed a severe headache, and about 2:00 p.m. had a convulsion, succeeded by stupor. She was brought to the hospital, with temperature 102.8°, pulse 72, blood pressure 150/98, comatose, vomiting, petechiae over the left forearm, stiff neck, blurring of the margins of the optic discs, heart moderately enlarged, occasional premature beats, no murmurs. The spinal fluid was bloody, and under increased pressure. After desensitization she was given intraspinally antimeningococcus serum, which produced accelerated serum sickness. She continued comatose until death, September 23. The temperature by September 20 had risen to 106.4°, blood nonprotein nitrogen was 42 mgm. per cent. The blood count was normal except for a leukocytosis (18,500 to 15,500 with 96 to 77 per cent polynuclears). The urine

contained a slight quantity of albumin, occasional red blood cells and granular casts, specific gravity up to 1.026. The blood pressure was 150 to 178/85 to 100 until the last day, when it fell to 110/86.

*Necropsy.* The right kidney weighed 125, the left 150 grams. The external surfaces of both were smooth and there were no scars in the cortex of either. The major renal vessels appeared to be free of atheroma. There was no evidence of pelvic or ureteral dilatation and infection.

A small hemorrhagic infarct was seen in the lower pole of the left kidney and several similar lesions of small size were noted in the spleen. Massive hemorrhages and tissue necrosis were found in both frontal lobes of the brain, which was the site of an intraventricular and sub-arachnoid hemorrhage.

The heart was not hypertrophied and weighed but 325 grams, whereas the body weight was 81 kilos. There was no evidence of passive congestion in the viscera.

*Microscopic.* Except for cloudy swelling the epithelium of the renal tubules was intact, and the lumina contained no casts. There were few scars in the cortex and pyramids and only an occasional glomerulus was completely hyalinized. On the other hand, in numerous glomeruli could be seen some degree, slight to moderate, of fibrin deposition in capillary tufts and thickening of afferent arterioles. Medial thickening was also noted in many of the larger renal vessels, but never to a very marked extent. The pelvic mucosa was not altered in appearance and the submucosa was free of cellular exudate.

#### Case 58750

Born 1894. Patient was first seen in the prenatal clinic January 25, 1927, three months pregnant, with blood pressure 170/110 and minimal albuminuria. She reported that her first pregnancy, in 1926, had terminated spontaneously in stillbirth at 7 months, after a period of vomiting, kidney trouble and edema of the legs. She was brought into the hospital, but refused treatment. The 1927 pregnancy and another in 1929 terminated, like the first, in spontaneous abortions at 7 months. The toxemic symptoms, however, became more severe with each pregnancy. After the last, she remained reasonably well until December 1931, when she began to suffer increasingly from dyspnea on exertion and orthopnea. About Christmas, edema began in the ankles and gradually spread to involve the legs, thighs and trunk. About the middle of January 1932, her right leg became infected and at about the same time her vision began to deteriorate. January 30 or 31, she became delirious. She entered the hospital, February 2, restless, disoriented, with marked dyspnea and orthopnea, temperature 99°, pulse 90, blood pressure 228/134, advanced retinal sclerosis, tongue and throat dry, raw and coated with yellow exudate, signs of pleural effusions and râles at the bases of both lungs with wheezes and rhonchi above, heart and liver greatly enlarged, massive edema of the lower extremities and trunk, ulcerations on both shins and cellulitis of the right leg, slight anemia, high leukocytosis



(24,700 with 92 per cent polynuclears), urine containing a little albumin and occasional leukocytes, blood non-protein nitrogen 64 mgm. per cent, serum protein 6.32 per cent, with albumin 3.27. She did not improve under treatment and died February 4. The temperature never exceeded 100.4°.

*Necropsy.* There was marked subcutaneous edema of both lower extremities, and in the right leg, in addition, multiple superficial ulcers from which exuded purulent material. About 100 cc. of thin, clear fluid were present in the peritoneal cavity, 300 cc. in the left and 1075 cc. in the right thoracic cavity, which also contained fibrin. The liver and both lungs showed evidence of marked chronic passive congestion.

The left kidney weighed 125 and the right 175 grams. Their external surfaces were finely granular and their cortices diffusely scarred. There was no apparent dilatation of the calices, pelves and ureters. The renal arteries were not appreciably thickened.

There was rather marked hypertrophy and dilatation of the heart which weighed 625 grams (body weight 83 kilos). The valves were normal.

*Microscopic.* There were numerous small scars in the renal cortex. Many tubules were small and atrophic but others were greatly dilated. The epithelium of the latter was frequently swollen and granular. In some of the tubules the epithelium was necrotic and desquamated; it often lay mingled with polymorphonuclear leukocytes in the lumina of the collecting tube. Completely hyalinized glomeruli were present as well as some which were atrophied and reduced to a few capillary loops. Many glomeruli were hypertrophied and filled their spaces completely. Very often glomeruli were seen

whose capillary loops were replaced with a pink-staining material like fibrin, the glomerular spaces containing small numbers of leukocytes, the walls of the afferent arterioles greatly thickened and the lumina partially or completely occluded (Figure 11). These changes resembled in every way those seen in so-called malignant nephrosclerosis. Other small arterioles in addition to the vasa afferentia had concentrically thickened walls with greatly narrowed or completely occluded lumina (Figure 12). Similar changes were seen throughout the body.

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# RENAL PHYSIOLOGY IN LOBAR PNEUMONIA<sup>1</sup>

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Previous studies on renal physiology during acute pneumococcus pneumonia have yielded rather discordant results. In general a hyperfunction has been reported, but this phenomenon has not always been observed. Lewis (1), McIntosh and Reimann (2) and Goldring (3) found an apparent increased functional capacity of the kidney during pneumonia. Rackemann, Longcope and Peters (4) and Frothingham (5) reported that there was neither impairment of function nor hyperfunction. On the other hand, Tileston and Comfort (6) Schwartz and McGill (7) and Bookman (8) reported definite evidence of renal impairment. Interest has been stimulated in the relation of the pneumococcus to renal pathology by the work of Blackman (9) and his coworkers on the association of this organism with the nephrotic syndrome. Rake (10) and Seegal (11) have reported on the incidence of nephritis in pneumococcus infection while Winkenwerder, McLeod and Baker (12) have tabulated the incidence of pneumonia preceding nephritis. In a survey of the literature by Rake, the pneumococcus was second to the streptococcus as a bacterial incitant of Bright's disease, while Winkenwerder, McLeod and Baker found that pneumonia was the type of infection observed at the onset of 6.5 per cent of patients in their series with Bright's disease. Seegal, however, observed but seven cases of nephritis in 1004 cases of pneumonia. The reasons for the variations in these statistics are not clear.

The present series of observations was undertaken to determine the immediate and delayed effect of pneumococcus pneumonia on renal function and to gain some understanding of the changes in renal physiology accompanying the period of febrile illness and also convalescence.

The primary mode of approach to estimation of renal function was through the urea clearance test and estimations of protein excretion in the urine. These two procedures could be carried on without

requiring too great cooperation of the patient and were independent of the treatment regime. Sediment counts were done on fresh morning specimens as an adjunct to the above tests. Other more elaborate tests were not done, as in many instances the patient was too ill to be disturbed.

## METHOD OF STUDY

Twenty-eight patients admitted consecutively to the Rockefeller Institute for treatment of pneumonia were observed over varying periods of time, in some instances as long as 6 months. On admission a urea clearance test was done; the following day a sediment count was performed. Urea clearances were repeated every three days until crisis, when practicable, and were always done on the day of crisis. During the acute illness 12-hour clearances were done unless the patient was incontinent, when the periods were shortened to those over which urine collection was complete. Another urea clearance was done 5 days post-crisis and at the time of discharge. Following discharge, urea clearances were done on the patients in the Outpatient Clinic once a month until renal function had been normal for two months. Sediment counts were done on the first day after crisis and on the sixth day after crisis. Quantitative estimations of urinary protein by the method of Shevky and Stafford (13) were done daily during the febrile period and as long thereafter as protein was present. Blood pressure determinations were done daily, and retinal examinations were made on each patient during the acute illness as well as in convalescence. Urea clearances were done more frequently than noted above whenever the results were outside the usual normal range. The patients were studied without reference to the type of pneumococcus infections or method of treatment. The bacteriological findings and treatment of the disease in these patients are shown in Table I. None of the patients gave any history of preexisting renal disease. In none of the patients was any retinopathy noted nor was there any marked sclerosis

<sup>1</sup> Read by title at the meeting of the American Society for Clinical Investigation, in Atlantic City, May, 1936.

TABLE 1

Summary showing number of patients with each type of pneumococcus

Pneumococcus type	Patient	Sex	Age	Serum therapy	Bacteremia	Outcome
			years			
I	A. B.	M.	17	+	—	Recovered
	M. A.	F.	18	+	+	Recovered
	C. E.	M.	15	+	—	Recovered
	P. T.	M.	17	+	+	Recovered
	E. M.	F.	29	+	—	Recovered
	H. Y.	F.	40	+	—	Recovered
	R. L.	F.	42	+	—	Recovered
II	A. K.	M.	30	+	—	Recovered
	S. M.	M.	56	+	—	Recovered
III	P. M.	M.	21	—	—	Recovered
	M. S.	F.	48	—	—	Recovered
	H. N.	F.	55	—	—	Recovered
	M. C.	M.	56	—	—	Recovered
	H. B.	M.	65	—	—	Recovered
	N. H.	F.	29	—	—	Recovered
	E. W.	M.	45	—	—	Died
V	E. C.	F.	10	—	—	Recovered
	O. S.	M.	39	—	—	Recovered
VII	O. K.	F.	22	—	—	Recovered
	J. T.	M.	28	+	—	Recovered
	M. B.	M.	20	—	—	Recovered
VIII	H. F.	M.	35	—	—	Recovered
	A. I.	M.	52	—	—	Recovered
IX	G. Z.	F.	49	—	—	Recovered
Unclassifiable	D. B.	F.	10	—	—	Recovered
	S. M.	F.	34	—	—	Recovered
	N. G.	M.	36	—	—	Recovered
B. Friedländer Type C	N. G.	M.	50	—	—	Died

of peripheral vessels. Blood cultures were done routinely on admission and were negative unless otherwise noted. The number of patients with each type of pneumococcus infection is summarized in Table I. The data on the two patients who died are shown in Table II-E.

#### RESULTS

*Urea clearance.* Renal function during the febrile period before crisis as revealed by the urea clearance test varied inversely as the age of the patient. This is demonstrated in Figure 1 and Tables II-A to II-E. During the febrile period the average urea clearance for eleven patients in the age group 10 to 29 years was elevated to 147 per cent of normal. The average for four pa-

tients 30 to 39 years was 111.8 per cent of normal; of five patients 40 to 49 years, 97.7 per cent normal; and of five patients 50 to 65 years, 82.6 per cent of normal. Thus it would appear that renal function during the pre-critical period of the disease may be elevated, may be unchanged, or may be slightly impaired, and that the level is in general a function of the age of the patient. The clearances done immediately after crisis and while the patient was still in the hospital do not show a rapid return to normal. On the contrary, the clearance in the younger group remained elevated for approximately a month after the onset of the disease. At no time during the acute illness did any of these younger patients have a urea clearance below 110 per cent of normal. In fact, all of the clearance figures at this time were 130 per cent or above, except in three instances shown in Figure 1. In each of these cases the clearance was done before fluid equilibrium had been established, and it was felt that the lower values so obtained (110.5, 113 and 113 per cent respectively) could be explained in part on this basis. Furthermore, during the first 20 days of the disease a majority of the urea clearance figures were elevated to 130 per cent or above except in 3 additional instances (Cases E. M., C. E., and N. H.—11th, 14th, and 15th days of disease). Fluid equilibrium had been established in these patients, however, and the reason for the lack of hyperfunction was not apparent.

In the age group 30 to 40 years, there was only moderate hyperfunction during the first ten days of the disease, and again in no instance was hypofunction noted. The number of patients in this group, however, is too small to warrant any conclusions.

In the age group 40 to 50 years, there was a definite absence of any increase of the urea clearance during the acute and convalescent periods of the illness. The clearance returned to an average value for this group in about three months. It is interesting to note that in this group of patients there is an apparent hyperfunction late in convalescence, occurring during the third month after onset, and that it is only after ninety days from onset that normal clearances are again observed. It was in this group that the lowest clearance was noted (first clearance 10.3 per cent on Case

H. Y.). The reason for this low value was not apparent.

In the oldest group there is no definite shift in the clearance below normal minimum, but the values during the acute illness are in general somewhat below normal average. No secondary variations in clearance values were noted in this group.

*Blood urea nitrogen.* The blood urea nitrogen in all of the patients at the time of admission was

that the patient was dehydrated. With restoration of fluids normal function followed, extra renal factors playing a predominant rôle.

*Proteinuria.* Contrary to expectation, the amount of protein lost in the urine by these patients during the febrile period was negligible. The majority of patients excreted less than 0.1 gram of protein per day throughout their entire hospital stay. In only one instance (E. C.) did

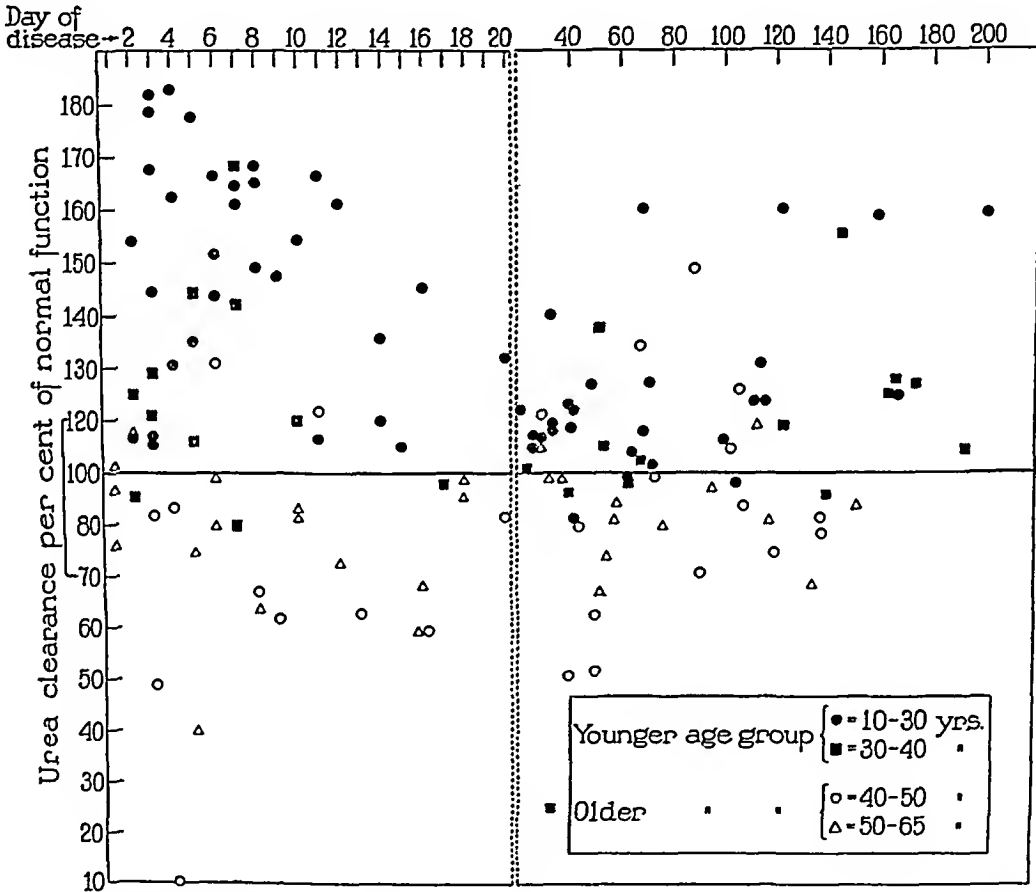


FIG. 1. SCATTER CHART SHOWING RELATION OF UREA CLEARANCE CHANGES TO DAY OF DISEASE.

within normal limits except for two patients (H. Y. and S. M.). The clearances, however, on admission, differed widely. In both exceptions noted above the blood urea nitrogen had dropped to normal five days later. In the patient H. Y. (Table II-C) there is a discrepancy between the clearance and the expected and observed blood urea nitrogen. Why this blood urea nitrogen should be lower than expected with a function of 10 per cent is not clear, unless it mirrors the fact

the protein excretion become significant, and in this patient the excretion never exceeded 3.0 grams per day and lasted for only four days. The early administration of adequate amounts of fluid to these patients seemed to depress protein excretion.

*Sediment counts.* In no single instance was the number of red blood cells excreted in 12 hours greater than the normal values defined by Addis. The difficulties of doing red blood cell counts on



# RENAL PHYSIOLOGY IN LOBAR PNEUMONIA

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TABLE II—Continued

PATIENTS IN LOBAR PNEUMONIA														
TABLE II—Continued														
Patient	Age	Urea clearance				Sediment and protein in 12-hrs. urine (no fluid restriction)								
		Day of dis- ease	Day before or after crisis	Blood urea nitrogen	Urea clear- ance, average normal	Day of dis- ease	Vol- ume in 12 hours	pH	Sediment				Proteinuria	
									R. B. C.	Casts		Total number in 12 hours		
										Total number in 12 hours	Hya- line			
	years			mgm. per cent	per cent		cc.				per cent	per cent	grams per liter	grams per 12 hours
A. PATIENTS 10 TO 29 YEARS—(Continued)														
J. T. ♂	28	2 6 11 16 43 105	-6 -2 +3 +8 +35 +97	15.97 15.90 10.88 10.88 9.33 10.47	113.5 151.5 166.4 145.0 82.0 96.7	3 8 12 17	370 950 442 690	6.0 7.0 5.0	0 0 45,000 20,000	20,000 70,000 8,000 50,000	100 100 50 100	50	0.07 <0.07 <0.07 <0.07	0.02
E. M. ♀	29	3 5 11 34	-2 0 +6 +29	7.15 9.44 12.20 7.01	113.6 177.6 113.6 118.4	4 6 13	950 430 1,190	7.0 7.0 7.0	0 0 0	30,000 45,000 30,000	100 66 100	33	<0.07 <0.07 <0.07	
T. H. ♀	29	4 10 15 42 72	-4 +2 +7 +34 +64	8.40 5.66 11.95 7.46 7.36	182.4 154.2 110.3 122.0 103.0	5 10 15	220 550 520	4+ 8.0 7.0	250,000 0 0	185,000 0 30,000	60 100	40	<0.07 <0.07 <0.07	
B. PATIENTS 30 TO 39 YEARS														
♂	30	3 7 54	0 +4 +51	19.46 16.00 16.32	120.6 142.0 109.9	3 7	380 359	5.0 7.0	9,000 0	18,000 6,000	100 100		0.10 <0.07	0.04
♂	35	2 5 10 52 121 140 174	-2 +1 +6 +48 +117 +136 +170	18.96 11.82 11.24 8.12 6.66 7.06 9.21	91.2 144.1 120.0 138.1 145.0 91.9 126.9	3 7 10	540 910 920	7.0 5.0 6.0	0 0 0	20,000 55,000 60,000			0.28 <0.07 <0.07	0.14
	36	5 17 63 123	-3 +9 +54 +114	19.38 11.69 11.49 13.46	111.3 95.8 98.1 119.0	5 16	407 610	5.0 7.0	16,000 0	220,000 180,000	60 100	40	0.50 <0.07	0.20
	39	2 7 24 68 165	-5 0 +17 +61 +158	15.17 33.13 11.43 10.61 15.97	125.0 79.0 101.6 104.6 128.3	9 28	830 700	4+ 6.0	0 0	370,000 20,000	60 100	40	<0.07 <0.07	
34		3 7 33 40 145 164 194	-3 +1 +27 +34 +139 +158 +188	8.71 6.24 26.43 6.60 7.59 9.50 11.36	129.5 168.0 25.1 92.8 155.3 125.0 108.9	3 8 34	215 550 360	4+ 6.0	30,000 50,000 0	22,000 85,000 0	100 100		2.6 <0.07 <0.07	0.46

## LEE E. FARR AND T. J. ABERNETHY

TABLE II—Continued

TABLE II—Continued																
Patient	Age	Urea clearance				Sediment and protein in 12-hrs. urine (no fluid restriction)										
		Day of dis- ease	Day before or after crisis	Blood urea nitrogen	Urea clear- ance, average normal	Day of dis- ease	Vol- ume in 12 hours	Sediment				Proteinuria				
								pH	R. B. C.	Casts						
										Total number in 12 hours	Total number in 12 hours	Hya- line	Gran- ular	grams per liter	grams per 12 hours	
	years			mgm. per cent	per cent		cc.					per cent	per cent			
C. PATIENTS 40 TO 50 YEARS																
H. Y. ♀	40	4 9 20 43 73 109 138	0 +5 +16 +39 +69 +105 +124	39.45 20.25 10.40 8.29 6.74 7.92 5.99	10.3 62.1 82.1 79.5 98.6 86.8 82.3	7	720	5.0	200,000	500,000	34	66	<0.07			
R. L. ♀	42	4 8 51 105 138	0 +4 +47 +101 +134	9.02 14.63 9.65 13.30 9.58	87.0 66.7 63.0 110.0 78.6	9	312	5.0	60,000	16,000	100		<0.07			
M. S. ♀	48	3 6 13 30 64 87 107	-3 0 +6 +23 +57 +80 +100	12.48 9.90 19.35 7.64 12.27 13.35 10.26	83.5 131.1 63.0 121.2 134.2 147.8 126.0	3 7 12	305 360 710	4+ 8.0 5.0	0 15,000 0	12,000 35,000 40,000	100 50 100	50	0.21 <0.07 <0.07	0.07		
G. Z. ♀	49	3 11 16 40 51 76 91 120	-6 +2 +7 +31 +42 +67 +82 +111	21.28 10.59 13.54 9.32 14.51 12.09 16.99 17.08	49.0 122.7 59.7 51.3 51.6 70.5 74.9	3 18	210 492	4+ 4+	0 0	350,000 0	10	90	0.43 <0.07	0.08		
D. PATIENTS 50 YEARS AND OVER																
A. I. ♂	52	1 6 18 58 95	0 +5 +17 +57 +94	14.11 11.53 12.85 9.84 10.94	101.2 80.2 92.3 80.5 95.2	2 7	330 660	7.0 5.0	0 0	0 0						
H. N. ♀	55	2 6 12 16 26 38 77	-3 +1 +7 +11 +21 +33 +72	11.40 13.15 16.29 19.90 11.93 11.04 6.82	115.6 98.3 72.7 59.9 109.9 98.6 80.0	4 12	900 500	6.0 4+	50,000 50,000	1,400,000 50,000	25 100	75	<0.07 <0.07	0.86 <0.07	0.78	
M. C. ♂	56	1 8 18 59 152	-6 +2 +12 +53 +146	16.89 24.48 15.31 18.47 19.54	75.9 63.5 98.2 89.3 87.5	3 19	375 650	4+ 6.0	25,000 0	45,000 18,000	75 100	25	0.14 <0.07	0.05		

TABLE II—Continued

# RENAL PHYSIOLOGY IN LOBAR PNEUMONIA

TABLE II—Continued

Patient	Age	Urea clearance				Sediment and protein in 12-hrs. urine (no fluid restriction)									
		Day of disease	Day before or after crisis	Blood urea nitrogen	Urea clearance, average normal	Day of disease	Volume in 12 hours	pH	Sediment				Proteinuria		
									R. B. C.	Casts					
										Total number in 12 hours	Total number in 12 hours	Hyaline per cent	Granular per cent	grams per liter	grams per 12 hours
years				mgm. per cent	per cent		cc.								
D. PATIENTS 50 YEARS AND OVER—Continued															
S. M. ♂	56	5 10 16 31 55 113 135	-4 +1 +7 +22 +46 +104 +126	61.40 17.08 17.96 10.95 12.47 12.14 15.88	24.3 82.5 69.0 97.8 73.5 119.1 68.2	8 13 18	633 890 590	5.0 7.0 6.0	0 0 180,000	20,000 30,000 18,000	100 100 100			0.14 <0.07 <0.07	0.08
H. B. ♂	65	1 5 10 52 119	-2 +3 +8 +50 +117	11.28 10.52 13.72 10.06 11.72	93.0 74.8 83.8 67.3 81.5	6 9	575 790	8.0 6.0	16,000 0	32,000 0	100			<0.07 <0.07	
E. PATIENTS WHO DIED															
E. W. ♂	45	3 5		22.13 21.91	70.6 109.2	4	285	4+	120,000	3,250,000	0	100		4.82	1.37
L. G. ♂	50	3		30.5	50.1	4	480	4+	0	2,000,000	0	100		0.72	

small samples of dilute urines must be borne in mind when interpreting these data. Casts, however, were uniformly increased in number with the hyaline type predominating. Urinary excretions were extremely low.

tween fluid intake and the amount of fluid excreted.

small samples of dilute urines must be borne in mind when interpreting these data. Casts, however, were uniformly increased in number with the hyaline type predominating. When the patients were extremely ill the proportion of granular casts increased. In the two patients who died, the differential cast count revealed 100 per cent granular casts with a large number of casts excreted in both instances.

*Estimations of blood pressure.* There was no significant alteration in the blood pressure accompanying the alteration in the urea clearance. In general, the blood pressure tended to be a little below normal during the febrile period of the disease, returning to the usual normal levels during convalescence.

*Additional laboratory findings.* Daily measurements of fluid intake and output were made on these patients and no deviations from the expected relations were noted. There was no relation between fluid intake and clearance, or be-

tween fluid intake and the number of observed formed elements in the urine. Some of the patients were given sodium chloride in therapeutic doses. This had no demonstrable effect on the urea clearance in these patients.

#### *Relation of renal physiology to pneumococcus type and therapy*

There was no demonstrable difference between the effects of different pneumococcal types on renal function. Nor did the early and effective use of therapeutic sera have any demonstrable effect on the renal function during the acute illness and also during serum disease. No effect was visible when treated and untreated patients in the same age groups were compared. It is possible that statistical analysis of a larger number of cases might show some correlation between pneumococcus type and changes in renal function. If any connection was present in our cases, however, it was quite obscured by the more marked



effects of age and increased lability of the renal function.

#### DISCUSSION

The outstanding revelation of these observations seems to be a decrease of renal resiliency with increasing age. The failure of older patients to develop a high urea clearance seems to be a part of their general loss of physiological elasticity. It must be emphasized here that normal children do not show urea clearances which, per square meter of body surface, exceed those of adults. It has been our experience, and also that of Cullen, Nelson and Holmes (14), that the variation in normal children from the mean, designated as "100 per cent normal," follows the same pattern as in adults. However, a marked response to alteration of some physiological factors governing the urea clearance indicating a high degree of resiliency of renal function has been observed in young children with nephrosis (Farr (15)).

The effect of age on renal behavior in pneumonia may explain, in part, the differing conclusions of previous investigators on the effect of pneumonia on renal function.

Our observations indicate no significant renal damage caused by the hyperpyrexia or the toxemia of the disease. It seems likely that the albuminuria observed in this disease is more closely allied to the benign albuminuria noted after severe exercise or cold showers than to the serious albuminuria seen in Bright's disease.

The fact that the urea clearance did not return to normal promptly with the temperature makes it seem unlikely that the clearance elevation noted is related to increase in metabolism associated with fever.

Prompt response to serum therapy did not appear to hasten the return of the inflated clearance to normal.

That hyperpyrexia of itself does not increase the urea clearance was shown by Farr and Moen (16) who observed a *decrease* in the clearance initially when artificial pyrexia was induced. Some factor other than fever must cause the increase in urea clearance in pneumonia.

These studies shed no light on the mechanism by which Bright's disease may follow pneumonia, as none occurred in this group.

It seems possible that signs of renal irritation, such as red cell excretion and albuminuria, may be kept to a minimum by adequate administration of fluid.

Administration of salt to some of these patients had no appreciable effect upon the apparent renal function.

#### CONCLUSIONS

1. Renal physiological studies have been made upon 28 patients with lobar pneumonia.

2. The urea clearance during the disease shows a sharp difference between the older and younger patients. The patients under 40 years of age show a marked elevation of the urea clearance, persisting about one month, and independent of the type of pneumonia or therapy used. The older age group show little change in the urea clearance.

3. Observation of the urine sediment in all groups showed a strong tendency for all abnormal elements to disappear with adequate fluid balance.

4. No significant changes in blood pressure were associated with the clearance changes.

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# STUDIES OF THE CIRCULATION IN PERNICIOUS ANEMIA<sup>1,2</sup>

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One of the most important functions of the blood is to furnish oxygen to the tissues in amounts sufficient for their requirements. The human organism has available several compensatory adjustments to counteract alterations which interfere with the normal supply of oxygen. Of these compensatory mechanisms three of the most important are: (1) variation in the oxygen carrying capacity of the blood, that is to say in the quantity of hemoglobin; (2) variation in the quantity of oxygen removed from the blood as it passes through the capillaries; and (3) alteration in the minute-volume output of the heart. It was with the object of studying certain responses of the heart to a slowly developing decrease in the oxygen capacity of the blood, such as is found in patients suffering from pernicious anemia, that these observations were made. We have made, therefore, measurements of the cardiac output, the cardiac size, the venous pressure, the circulation time, the oxygen consumption, the vital capacity, the heart rate, and the blood pressure in five patients suffering from pernicious anemia. These measurements were made first during the anemic state and later during a remission induced by liver extract, when the count of the red blood cells and the hemoglobin values had reached more nearly normal levels. Although there are studies in the literature dealing with certain phases of the circulation, there are none in which the several functions mentioned above were observed simultaneously.

## METHODS

All observations were made in the morning while the patients were in a basal metabolic state. Measurements of the cardiac output were made by the acetylene method,

<sup>1</sup> An abstract of these studies was read before the Association of American Physicians, Atlantic City, May 5, 1936.

<sup>2</sup> This Paper is also Number 1 of the Series on Studies of the Circulation published from the New York Hospital and the Department of Medicine, Cornell University Medical College.

three samples of gas being taken as first recommended by Grollman (1), and by Grollman, Friedman, Clark and Harrison (2). During this measurement the patients were sitting in a steamer chair (angle 135 degrees) with the legs extended. They were trained beforehand to carry out the procedures. While the patient was at rest, the radial pulse was counted at intervals of five minutes. At the end of one-half hour the acetylene-air-oxygen mixture was rebreathed. Three samples of gas were taken during each rebreathing period for estimation of the arteriovenous oxygen difference. Three periods of rebreathing were carried out on each patient. Shortly afterward, the oxygen consumption was measured with a Benedict-Roth spirometer. After a short pause, the vital capacity was measured, and height and weight recorded. Then the patient rested again, now lying down. In succession, sufficient time being allowed between each procedure for the patient to return to a basal metabolic state, an electrocardiogram was taken, the arm to tongue circulation time recorded, the venous pressure estimated, and the blood pressure measured; finally an x-ray photograph of the heart was made at a distance of two meters.

The arm to tongue circulation time was estimated by the use of decholin: 5 cc. of a 20 per cent solution were injected rapidly (1 to 2 seconds) through an 18 gauge needle into an antecubital vein while the patient was lying quietly in the supine position. This was repeated in one and one-half minutes after the response to the first test had been elicited. The time was recorded from the beginning of the injection until the patient perceived the bitter taste. The injection time was also recorded, but since the response may come with a minimal amount of the drug, the time which we have used was taken from the start, rather than from the conclusion of the injection.

The venous pressure was measured by the direct method (3), using a large antecubital vein, the arm being placed on a level with the right auricle. The apparatus consisted of an L-tube of glass attached to a three-way stopcock, a syringe, and an 18 gauge needle. The apparatus was filled with a solution of sterile normal saline, a venepuncture performed, and the direct pressure readings recorded. Normal pressures with this apparatus range from 4.0 to 9.0 cm. of saline. The antecubital vein of one arm was reserved for the injection of decholin and of the other arm for the measurement of venous pressure. In subsequent measurements the vein was entered at the site first punctured.

X-ray photographs of the heart were taken with the patient in the standing position, in full inspiration, at

a distance of two meters.<sup>3</sup> Measurements of the cardiac area were carried out by the technique of Levy (4) and estimations of volume were made as recommended by Bardeen (5). The volumes recorded in Table I have not been multiplied by the constant which is in Bardeen's formula. This was done in order to make our observations comparable to those of Starr, Collins and Wood (13).

### OBSERVATIONS

*Case 1*, a white woman 39 years of age, was admitted to the hospital on February 6, 1935. For 6 months before admission she had experienced increasing weakness, dyspnea, palpitation, intermittent diarrhea, and a loss in weight of 25 kgm. Pallor and swelling of the ankles toward evening appeared during the last two months. On examination the patient was obese and quite pale. The heart was slightly enlarged (Table I). The rhythm was normal sinus mechanism. A systolic murmur was heard at the apex. Neither cyanosis nor dyspnea were observed. Râles were not heard at the lung bases. The liver and spleen were not palpable. Moderate pitting edema was present below the knees. Free hydrochloric acid was not present in the gastric contents after the administration of histamine. The patient was anemic and a blood smear showed changes typical of pernicious anemia.

On February 9, 1935, when the count of the red blood cells was 1,070,000, the various measurements recorded in Table I were made. On a regime of injections of liver extract<sup>4</sup> intramuscularly, 10 cc. daily for one week followed by biweekly injections of 10 cc. of this extract, together with reduced iron 1.5 grams by mouth, daily, the anemia became less severe, and on March 14, 1935, a second series of measurements was recorded (Table I).

*Case 2*, a white woman 54 years of age, was admitted to the hospital on March 2, 1935. She had first observed dyspnea and palpitation on exertion 11 years before admission. For two years she had experienced severe epistaxis and was then told for the first time that the blood pressure was elevated. Following a recurrence of epistaxis in December 1934, she suffered from increased fatigability and increasing dyspnea and palpitation on exertion, although she was still able to climb four flights of stairs. Two weeks before admission she became aware of a yellowish pallor of the skin. On examination the patient was obese. There was a pale icteric tint of the skin. The tongue margin was smooth. A firm nodule was felt in the isthmus of the thyroid, but tremor of the hands was not present. The heart was slightly enlarged (Table I). Regular sinus rhythm was present. A systolic murmur was heard at the apex. The second sound over the aortic area was louder than the second

pulmonic sound. The blood pressure was labile, varying during her stay in the hospital between 130 and 214 mm. of Hg systolic, and 80 to 126 mm. of Hg diastolic. Cyanosis, dyspnea, and edema were not observed. A few moist râles were heard at the lung bases at the time of admission. These disappeared during the first few days in bed. The liver and spleen were not palpable. There was a trace of albumin in the urine and occasional red blood cells were present in the centrifuged sediment. The urea clearance was 63 per cent of normal. The gastric contents did not contain free HCl after the administration of histamine. A blood smear showed the changes typical of pernicious anemia. The following diagnoses were made: Pernicious anemia, arterial hypertension; normal sinus rhythm; obesity due to excess food; and non-toxic nodular goiter.

On March 8 when the count of the red blood cells was 1,190,000 the first series of measurements was made (Table I). The patient then received injections of liver extract intramuscularly, 10 cc., daily for one week followed by injections of 10 cc. of the extract every third day, together with 12 cc. of a 50 per cent solution of iron and ammonium citrate given by mouth daily. On this regime the count of the red blood cells rose to 3,200,000 on March 27, 1935, and the second series of measurements was recorded (Table I).

*Case 3*, a white male 66 years of age, was first admitted to the hospital on August 23, 1929, complaining of anorexia and weakness of ten weeks' duration. On examination, marked pallor was present. The count of the red blood cells was 1,110,000 and the estimation of the hemoglobin was 22 per cent (14.5 grams hemoglobin equivalent to 100 per cent). The blood smear showed changes typical of pernicious anemia. On a diet containing liver the patient improved and was discharged on September 12, the count of the red blood cells then being 2,180,000 and the estimation of the hemoglobin 41 per cent. Three months following discharge he stopped taking liver and felt well until three weeks prior to his second admission when he experienced weakness, diarrhea, tingling of the fingers and toes. He was readmitted to the hospital on February 15, 1935. The patient was a thin, pale, elderly male, who showed no dyspnea, cyanosis nor edema. The heart was not enlarged (Table I). Regular sinus rhythm was present. No murmurs were heard. Râles were not heard at the lung bases; the liver and spleen were not palpable. Neurological changes were not present. The gastric contents did not contain free hydrochloric acid after the administration of histamine, and a blood smear showed the changes typical of pernicious anemia.

On February 19 the count of the red blood cells was 1,410,000, and the first series of measurements was made (Table I). On a regime of injections of liver extract intramuscularly, 10 cc. daily for one week followed by biweekly injections of 10 cc. of this extract, together with 1.5 grams of reduced iron by mouth daily, the count of the red blood cells rose to 3,500,000 on March 9, 1935, and the second series of measurements was recorded (Table I)

<sup>3</sup> The authors are deeply indebted to the X-ray Department of the New York Hospital for their cooperation in this investigation.

<sup>4</sup> The extract which was given to all patients was prepared at the New York Hospital unless otherwise specified.

TABLE I  
Data on five patients suffering from pernicious anemia

Case, hospital number, age	Date	Body surface square meters	Oxygen consumption cc. per minute	Basal metabolic rate <sup>*</sup> per cent	Arterio-venous oxygen difference cc.	Cardiac output liters per minute	Cardiac output liters per sq. m. per minute	Heart rate per minute	Cardiac output cc. per beat	Cardiac area sq. cm.	Cardiac volume cc.	Measurements of x-ray photographs of the heart			Arterial pressure mm. Hg	Circulation time seconds	Venous pressure cm.	Vital capacity cc.	Left ventricular work kilogram-meters per minute	Left ventricular work gram-meters per beat	Red blood cells millions	Hemoglobin (Sahli) <sup>†</sup> per cent
												Transverse diameter of heart	Internal diameter of aortic orifice of heart	Cardio-thoracic ratio								
Case 1, A. N. .... No. 81501 30 years, ♀	Feb. 9, 1935	1.83	200.0	+10	45.4	5.93	3.21	90	61.8	132.0	1306	cm.	cm.	0.53	94/50	8.8	7.8	2500	5.89	90.5	1.07	27
Case 2, A. D. .... No. 81502 55 years, ♀	Mar. 14, 1935	1.80	248.4	+9	57.8	4.30	2.31	74	58.0	120.7	1208	14.2	28.0	0.51	110/72	13.6	8.9	2800	5.40	74.2	3.40	76
Case 3, J. J. .... No. 81503 60 years, ♂	Mar. 8, 1935	1.75	248.0	+24	55.0	4.53	2.60	90	50.2	118.5	1175	14.0	27.8	0.50	134/80	7.6	11.1	2000	0.58	73.1	1.10	42
Case 3, J. J. .... No. 81503 60 years, ♂	Mar. 27, 1935	1.73	269.0	+0	64.0	3.23	1.99	84	38.4	118.9	1183	14.7	27.0	0.53	214/126	10.8	9.5	2700	7.40	88.8	3.20	69
Case 3, J. J. .... No. 81503 60 years, ♂	Feb. 10, 1935	1.59	228.0	+24	43.2	5.28	3.52	82	64.4	109.1	1040	11.6	27.3	0.43	90/50	12.5	7.7	2650	5.93	91.3	1.41	35
Case 4, L. W. .... No. 81503 49 years, ♂	Mar. 9, 1935	1.51	205.8	+12	59.3	3.47	2.30	88	59.8	117.5	1160	11.9	27.0	0.43	149/70	16.0	7.4	3150	4.95	85.4	3.50	72
Case 4, L. W. .... No. 81503 49 years, ♂	Dec. 20, 1931	1.70	291.0	+20	44.4	5.83	3.40	88	68.8	121.5	1229	14.7	28.8	0.51	191/50	10.7	6.5	2700	0.10	70.0	1.35	45
Case 4, L. W. .... No. 81503 49 years, ♂	Jan. 14, 1935	1.70	210.7	+1	53.9	4.93	2.40	80	51.9	123.9	1257	14.5	29.2	0.59	128/80	11.9	8.0	2000	5.77	72.1	3.70	58
Case 5, A. M. .... No. 81509 45 years, ♀	Feb. 13, 1935	1.45	291.0	+15	49.6	4.05	2.80	80	50.9	115.5	1132	13.0	26.6	0.49	108/60	13.4	4.5	3000	4.02	57.7	2.85	92
Case 5, A. M. .... No. 81509 45 years, ♀	Mar. 12, 1935	1.47	181.0	+4	63.5	2.99	2.90	72	40.3	111.8	1076	12.8	28.5	0.45	100/80	14.8	7.5	2900	4.74	95.3	3.80	75

\* According to Normograms of W. M. Boothby and J. Berkson, August 1935.

† The volumes have not been multiplied by the constant in Bardeen's Formula, see text, p. 432.

‡ 14.5 grams hemoglobin equivalent to 100 per cent.

a distance of two meters.<sup>3</sup> Measurements of the cardiac area were carried out by the technique of Levy (4) and estimations of volume were made as recommended by Bardeen (5). The volumes recorded in Table I have not been multiplied by the constant which is in Bardeen's formula. This was done in order to make our observations comparable to those of Starr, Collins and Wood (13).

#### OBSERVATIONS

*Case 1*, a white woman 39 years of age, was admitted to the hospital on February 6, 1935. For 6 months before admission she had experienced increasing weakness, dyspnea, palpitation, intermittent diarrhea, and a loss in weight of 25 kgm. Pallor and swelling of the ankles toward evening appeared during the last two months. On examination the patient was obese and quite pale. The heart was slightly enlarged (Table I). The rhythm was normal sinus mechanism. A systolic murmur was heard at the apex. Neither cyanosis nor dyspnea were observed. Râles were not heard at the lung bases. The liver and spleen were not palpable. Moderate pitting edema was present below the knees. Free hydrochloric acid was not present in the gastric contents after the administration of histamine. The patient was anemic and a blood smear showed changes typical of pernicious anemia.

On February 9, 1935, when the count of the red blood cells was 1,070,000, the various measurements recorded in Table I were made. On a regime of injections of liver extract<sup>4</sup> intramuscularly, 10 cc. daily for one week followed by biweekly injections of 10 cc. of this extract, together with reduced iron 1.5 grams by mouth, daily, the anemia became less severe, and on March 14, 1935, a second series of measurements was recorded (Table I).

*Case 2*, a white woman 54 years of age, was admitted to the hospital on March 2, 1935. She had first observed dyspnea and palpitation on exertion 11 years before admission. For two years she had experienced severe epistaxis and was then told for the first time that the blood pressure was elevated. Following a recurrence of epistaxis in December 1934, she suffered from increased fatigability and increasing dyspnea and palpitation on exertion, although she was still able to climb four flights of stairs. Two weeks before admission she became aware of a yellowish pallor of the skin. On examination the patient was obese. There was a pale icteric tint of the skin. The tongue margin was smooth. A firm nodule was felt in the isthmus of the thyroid, but tremor of the hands was not present. The heart was slightly enlarged (Table I). Regular sinus rhythm was present. A systolic murmur was heard at the apex. The second sound over the aortic area was louder than the second

pulmonic sound. The blood pressure was labile, varying during her stay in the hospital between 130 and 214 mm. of Hg systolic, and 80 to 126 mm. of Hg diastolic. Cyanosis, dyspnea, and edema were not observed. A few moist râles were heard at the lung bases at the time of admission. These disappeared during the first few days in bed. The liver and spleen were not palpable. There was a trace of albumin in the urine and occasional red blood cells were present in the centrifuged sediment. The uric acid clearance was 63 per cent of normal. The gastric contents did not contain free HCl after the administration of histamine. A blood smear showed the changes typical of pernicious anemia. The following diagnoses were made: Pernicious anemia, arterial hypertension; normal sinus rhythm; obesity due to excess food; and non-toxic nodular goiter.

On March 8 when the count of the red blood cells was 1,190,000 the first series of measurements was made (Table I). The patient then received injections of liver extract intramuscularly, 10 cc., daily for one week followed by injections of 10 cc. of the extract every third day, together with 12 cc. of a 50 per cent solution of iron and ammonium citrate given by mouth daily. On this regime the count of the red blood cells rose to 3,200,000 on March 27, 1935, and the second series of measurements was recorded (Table I).

*Case 3*, a white male 66 years of age, was first admitted to the hospital on August 23, 1929, complaining of anorexia and weakness of ten weeks' duration. On examination, marked pallor was present. The count of the red blood cells was 1,110,000 and the estimation of the hemoglobin was 22 per cent (14.5 grams hemoglobin equivalent to 100 per cent). The blood smear showed changes typical of pernicious anemia. On a diet containing liver the patient improved and was discharged on September 12, the count of the red blood cells then being 2,180,000 and the estimation of the hemoglobin 41 per cent. Three months following discharge he stopped taking liver and felt well until three weeks prior to his second admission when he experienced weakness, diarrhea, tingling of the fingers and toes. He was readmitted to the hospital on February 15, 1935. The patient was a thin, pale, elderly male, who showed no dyspnea, cyanosis nor edema. The heart was not enlarged (Table I). Regular sinus rhythm was present. No murmurs were heard. Râles were not heard at the lung bases; the liver and spleen were not palpable. Neurological changes were not present. The gastric contents did not contain free hydrochloric acid after the administration of histamine, and a blood smear showed the changes typical of pernicious anemia.

On February 19 the count of the red blood cells was 1,410,000, and the first series of measurements was made (Table I). On a regime of injections of liver extract intramuscularly, 10 cc. daily for one week followed by biweekly injections of 10 cc. of this extract, together with 1.5 grams of reduced iron by mouth daily, the count of the red blood cells rose to 3,500,000 on March 9, 1935, and the second series of measurements was recorded (Table I).

<sup>3</sup> The authors are deeply indebted to the X-ray Department of the New York Hospital for their cooperation in this investigation.

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Data on five patients suffering from pernicious anemia

Case, hospital number, age	Date	Body surface square meters	Oxygen consumption cc. per minute	Basal metabolism rate* per cent	Arterio-venous oxygen difference cc.	Cardiac output liters per minute	Cardiac output cc. per beat	Cardiac area sq. cm.	Cardiac volume cc.	Measurements of x-ray photographs of the heart			Arterial pressure mm. Hg	Circulation time seconds	Venous pressure cm.	Vital capacity cc.	Left ventricular work kilogram-meters per minute	Left ventricular work gram-meters per beat	Red blood cells millions	Hemoglobin (Sahli) per cent
										Transverse diameter of heart	Inter-ventricular diameter of chest	Cardio-thoracic ratio								
Case 1, A. N., No. 88101, 30 years, ♀	Feb. 9, 1935	1.85	200.0	+19	45.4	5.03	61.8	132.0	1390	14.8	27.7	0.53	94/50	8.8	7.8	2500	5.80	60.5	1.07	27
Case 1, A. N., No. 88101, 30 years, ♀	Mar. 14, 1935	1.86	218.4	+9	57.8	4.30	58.0	120.7	1208	14.2	28.0	0.51	116/72	13.0	8.9	2800	5.49	74.2	3.40	79
Case 2, A. D., No. 90312, 55 years, ♀	Mar. 8, 1935	1.75	218.0	+24	55.0	4.62	50.2	118.5	1175	14.6	27.8	0.50	134/86	7.9	11.1	2696	6.58	73.1	1.10	42
Case 2, A. D., No. 90312, 55 years, ♀	Mar. 27, 1935	1.73	209.0	+6	64.9	3.23	38.4	118.0	1183	14.7	27.9	0.53	214/120	10.8	9.5	2700	7.40	88.8	3.20	66
Case 3, J. J., No. 89277, 69 years, ♂	Feb. 10, 1935	1.50	228.9	+24	43.2	5.23	64.4	109.1	1019	11.0	27.3	0.43	99/50	12.5	7.7	2850	5.93	61.3	1.41	35
Case 3, J. J., No. 89277, 69 years, ♂	Mar. 9, 1935	1.51	205.8	+12	69.3	3.47	59.8	117.5	1160	11.9	27.9	0.43	110/70	16.0	7.4	3150	4.95	85.4	3.50	72
Case 4, L. W., No. 81805, 49 years, ♂	Dec. 20, 1934	1.70	201.9	+20	44.4	5.88	63.8	121.5	1220	14.7	28.8	0.51	104/50	10.7	6.5	2700	9.16	70.0	1.35	45
Case 4, L. W., No. 81805, 49 years, ♂	Jan. 14, 1935	1.70	210.7	+1	53.9	4.98	51.0	123.9	1257	14.6	29.2	0.50	128/80	11.9	8.9	2900	5.77	72.1	3.70	58
Case 5, A. M., No. 81809, 45 years, ♀	Feb. 13, 1935	1.45	201.9	+15	40.6	4.05	50.6	115.5	1132	13.0	26.6	0.40	108/60	13.4	4.5	3900	4.02	57.7	2.85	62
Case 5, A. M., No. 81809, 45 years, ♀	Mar. 12, 1935	1.47	184.0	+4	93.5	2.90	40.3	111.8	1076	12.8	28.5	0.45	109/80	14.8	7.6	2900	4.74	95.8	3.80	75

\* According to Normograms of W. M. Boothby and J. Berkson, August 1935.

† The volumes have not been multiplied by the constant in Bardeen's Formula, see text, p. 432.

‡ 14.5 grams hemoglobin equivalent to 100 per cent.



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												Trans- verse diam- eter of left heart	Inter- ven- tricular diam- eter of chest	Cardio- thoracic ratio								
Case 1, A. N., No. 8810 30 years, ♀	Feb. 6, 1935 Mar. 14, 1935	1.85 1.80	200.0 218.4	+10 + 0	45.4 57.8	5.03 4.30	3.21 2.31	06 74	61.8 58.0	132.0 126.7	1306 1208	14.8 14.2	27.7 28.0	0.53 0.51	94/80 110/72	8.3 13.0	7.8 8.9	2500 2800	5.80 5.40	00.5 74.2	1.07 3.40	27 70
Case 2, A. D., No. 8812 55 years, ♀	Mar. 8, 1935 Mar. 27, 1935	1.75 1.73	218.0 200.0	+24 + 0	55.0 64.0	4.52 3.23	2.60 1.96	00 84	50.2 38.4	118.5 118.9	1175 1183	14.6 14.7	27.3 27.6	0.50 0.53	134/86 214/126	7.6 10.8	11.1 0.5	2600 2700	0.58 7.46	73.1 88.8	1.19 3.20	42 66
Case 3, J. J., No. 8927 60 years, ♂	Feb. 10, 1935 Mar. 9, 1935	1.50 1.51	228.0 205.8	+24 +12	43.2 59.3	5.28 3.47	3.52 2.30	82 58	64.4 50.8	109.1 117.5	1040 1160	11.0 11.9	27.3 27.6	0.43 0.43	96/80 140/70	12.5 16.0	7.7 7.4	2850 3150	5.03 4.95	61.3 85.4	1.41 3.50	35 72
Case 4, L. W., No. 81605 40 years, ♂	Dec. 20, 1934 Jan. 14, 1935	1.76 1.70	201.0 210.7	+20 + 1	44.4 53.6	5.88 4.05	3.40 2.40	88 80	63.8 51.0	121.5 123.0	1220 1257	14.7 14.6	28.8 29.2	0.51 0.50	104/80 128/86	10.7 11.6	6.5 8.0	2700 2900	6.10 5.77	70.0 72.1	1.35 3.70	45 58
Case 5, A. M., No. 81609 45 years, ♀	Feb. 13, 1935 Mar. 12, 1935	1.45 1.47	201.0 184.0	+15 + 4	40.6 63.5	4.05 2.90	2.80 2.00	80 72	50.0 40.3	115.5 111.8	1132 1076	13.0 12.8	26.6 28.5	0.40 0.45	103/86 100/80	13.4 14.8	4.5 7.6	3000 2900	4.02 4.74	57.7 65.8	2.85 3.80	62 75

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† The volumes have not been multiplied by the constant in Bardeen's Formula, see text, p. 432.

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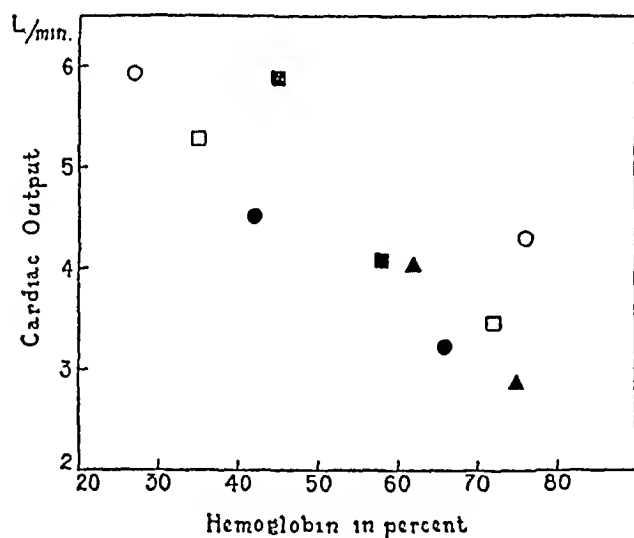


FIG. 3.

In this figure all observations of cardiac output of all patients are plotted against corresponding hemoglobin measurements. This discloses a correlation which appears to be linear, in that as the hemoglobin decreases the cardiac output increases. The symbols representing each patient are the same as those used in Figure 1.

cardiac rates are more rapid than when the red blood cells and hemoglobin have assumed more nearly normal values (Table I); the cardiac output decreases progressively as the hemoglobin values (Figure 3) and red blood cell counts rise (Figure 4) until normal values of minute-volume output are finally reached at hemoglobin concentration of approximately 70 per cent.

Calculations of the percentage oxygen utilization (Table II) showed that it was greater during

the anemic state than after the amount of hemoglobin increased, an observation to which Richards and Strauss (10) have already directed attention as one of the compensatory adjustments in anemia. In short, the percentage oxygen utilization varies inversely as the oxygen capacity (Table II) although the arteriovenous oxygen difference decreases in anemia.

TABLE II

*Data relating to percentage of oxygen utilization in 5 anemic patients*

Case number	O <sub>2</sub> capacity*		Oxygen utilization†	
	Before‡	After‡	Before‡	After‡
	volumes per cent	volumes per cent	per cent	per cent
1	5.4	15.2	83	38
2	8.4	13.2	65	49
3	7.0	14.4	61	41
4	9.0	11.6	49	47
5	12.4	15.0	40	43

\* 1 per cent hemoglobin (Table I) equivalent to 0.2 volume per cent oxygen capacity. This was assumed to be accurate enough for the deduction to be made from the data derived from the calculations.

† Percentage of oxygen utilization is the ratio between arteriovenous oxygen difference (Table I) and oxygen capacity.

‡ "Before" and "After" refer to the period of anemia and period of increased hemoglobin respectively.

In these patients, as the cardiac output increased the velocity of blood flow increased, a

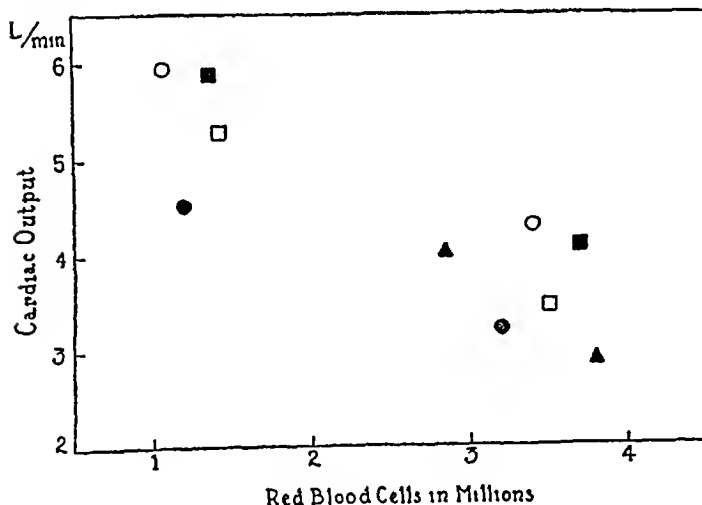


FIG. 4.

In this figure all observations of red blood cell counts of all patients are plotted against the corresponding cardiac output. A correlation similar to that demonstrated in Figure 3 appears. The symbols are the same as those in Figures 1 and 3.

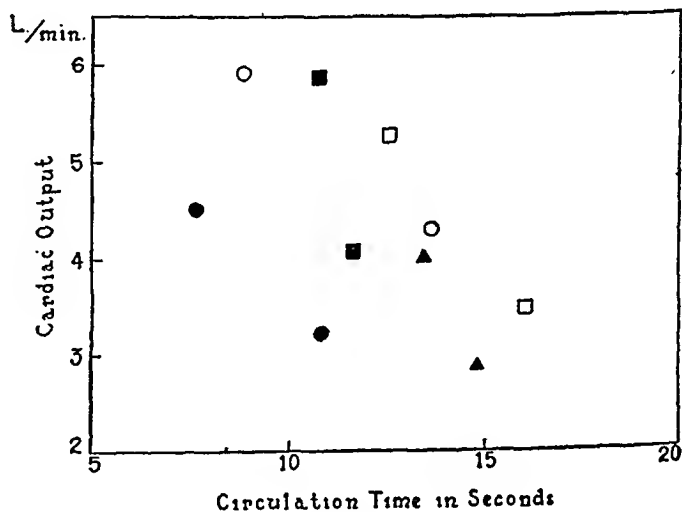


FIG. 5.

In this figure all observations of circulation time are plotted against cardiac output. This reveals a linear correlation in that as the cardiac output decreases the circulation time becomes longer. The symbols are the same as in Figure 1.

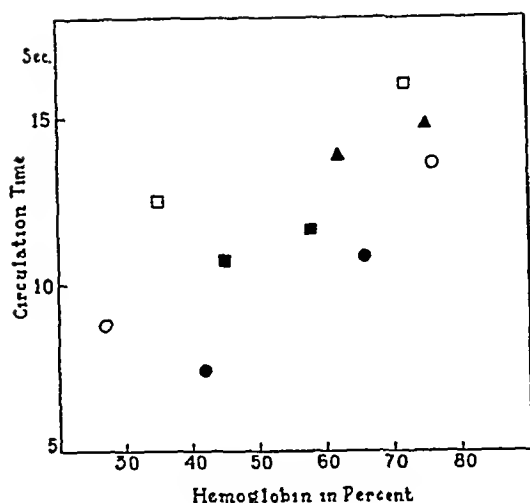


FIG. 6.

In this figure all observations of hemoglobin are plotted against circulation time. A linear correlation appears. As the hemoglobin increases the circulation time becomes longer. The symbols are the same as in Figure 1.

linear relationship being maintained. The greater the cardiac output, the shorter the circulation time (Figure 5). Moreover, there appeared a correlation between circulation time and quantity of hemoglobin; at low hemoglobin percentages the circulation time was short, only to become longer as the hemoglobin values increased (Figure 6).

These results are similar to those recorded in the studies of Blumgart, Gargill and Gilligan (14) relating to velocity of pulmonary blood flow in patients suffering from pernicious anemia. The increase in velocity is, however, of somewhat greater magnitude than that reported by Tarr, Oppenheimer, and Sager (15) who, also using decholin, found an average arm to tongue circulation time of 12 seconds in their anemic patients, as opposed to 13 seconds in their normal control series. Since the hemoglobin levels at which their observations were made are not given it is not possible to make an accurate comparison of their observation with ours.

The venous pressure showed no significant alterations except in the case of one patient (Case 2, Table I) who showed a slightly elevated pressure at the low hemoglobin levels which approached a more normal value as the hemoglobin concentration in the blood increased.

In all patients there was a rise in both the

systolic and diastolic levels of blood pressure as the hemoglobin increased; in three instances the increase in blood pressure was very striking. In short, although the heart put out an increased quantity of blood per minute and per beat in the presence of anemia the blood pressure was lower. The manner in which these factors contribute to the work of the heart is discussed later.

The vital capacity of the lungs was moderately reduced in four of the patients during the anemic state although no signs of congestive heart failure were present. We are unable to explain this satisfactorily but call attention to the suggestion offered by Blumgart, Gargill and Gilligan (14) that it may be related to the presence of an increased amount of blood in the lungs coincident with an increased rate of flow.

The electrocardiograms showed no significant alterations as the hemoglobin concentration in the blood increased.

Du Bois (16) has summarized data in the literature relating to the basal metabolic rate in anemia. Certain subjects with anemia show increased and others decreased basal metabolic rates. We have observed increased metabolic rates in each instance at low hemoglobin levels (Table I); as the hemoglobin content of the blood increased the oxygen consumption decreased and ap-

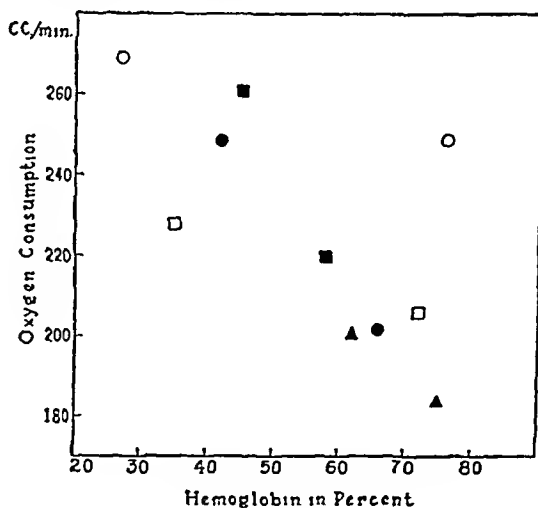


FIG. 7.

In this figure all observations of hemoglobin are plotted against oxygen consumption. A linear relationship is revealed, in that as the hemoglobin increases the oxygen consumption decreases. The symbols are the same as in Figure 1.

proached more nearly normal values (Figure 7). This result is similar to that growing out of the observations of Tompkins, Brittingham and Drinker (17) who approached this question in another manner. They found that the metabolic rates of patients suffering from pernicious anemia decreased following the transfusion of blood. It appears clear, therefore, both from our own observations and from those already recorded by others, that the oxygen consumption, in short, the basal metabolic rate, may be increased appreciably in the presence of pernicious anemia. A satisfactory explanation for this phenomenon has not yet been advanced. Our data do not permit us to make any positive contribution to this puzzling observation. They indicate, however, as will be shown later, that increased oxygen consumption is not a consequence of increased work of the heart, a notion which has been current up to the present.

That enlargement of the heart in anemic patients occurs as a consequence of both dilatation and hypertrophy is an idea that has been held for many years (18, 19, 7). Most of the conclusions have been based upon clinical and postmortem observations in cases of pernicious anemia. One patient reported by Ball (20) is of especial interest since roentgenological enlargement of the heart was observed during severe secondary anemia, and was followed by decrease in size when the blood count returned to normal. From these observations he concluded that enlargement was due to dilatation rather than hypertrophy. In the case of three patients in the series now being reported, the heart was moderately enlarged or at the upper limits of normal, only two being well within normal limits. That observations made after improvement failed to show significant change in size of the heart may be due in part to the relatively short time covered by observations of each patient.

The cause of cardiac enlargement found in the presence of anemia has been subject to controversy. One notion (11) regards it as a consequence of increased activity of the heart; another (7) attributes it to lack of oxygen in the myocardium. Because of these notions it appeared of interest to ascertain the amount of work done by the heart in patients suffering from pernicious

anemia. Work may be calculated by making use of the formula (21)

$$W = QR + \frac{wV^2}{2g}$$

in which  $W$  equals work done per minute, or per beat;  $Q$  equals volume of blood expelled per minute, or per beat;  $R$  equals mean arterial blood pressure in mm. of Hg  $\times 13.6$ ;  $V$  equals velocity of blood at aorta;  $w$  equals weight of blood;  $g$  equals acceleration due to gravity. The last part of the formula,  $\frac{(wV^2)}{2g}$ , has been omitted in order to make our results comparable to those of Starr et al. (13). By substituting values in this formula, we have calculated the work of the left ventricle both per beat and per minute. Since there is evidence (22) that the work of the right ventricle bears a constant relationship to that of the left, we have concerned ourselves only with the latter. The work per beat done by the left ventricle was found less during the period of increased cardiac output at low hemoglobin levels, than later when the output was lowered and the amount of hemoglobin more nearly normal (Table I).

Taking into account the increased heart rates found in the anemic state, as is done in computing the work per minute, does not reveal increased work in the presence of anemia. This is due for the most part to the considerable increase in the mean arterial pressure which is found at the higher levels of hemoglobin. Since, therefore, the work of the heart appears to be within normal limits there seems to be no point in retaining the notion which is current, that enlargement of the heart in anemia is a consequence of increased cardiac work; on the other hand, by exclusion it lends credence to the view that myocardial anoxemia may be the stimulus which initiates enlargement. Likewise, since we have failed to find the work of the heart increased in these patients, this mechanism does not account for the increased basal metabolic rate.

Stewart and Cohn (23) expressed the opinion that the explanation of the increase in cardiac output with decrease in cardiac size as a consequence of giving digitalis to patients suffering from congestive heart failure, was to be found in Starling's "Law of the Heart" (24). Further-

more, Starr and his associates (12, 13) have presented since then, data indicating that this "Law" applies to basal cardiac work in human beings, as well as to the heart-lung preparation. For it

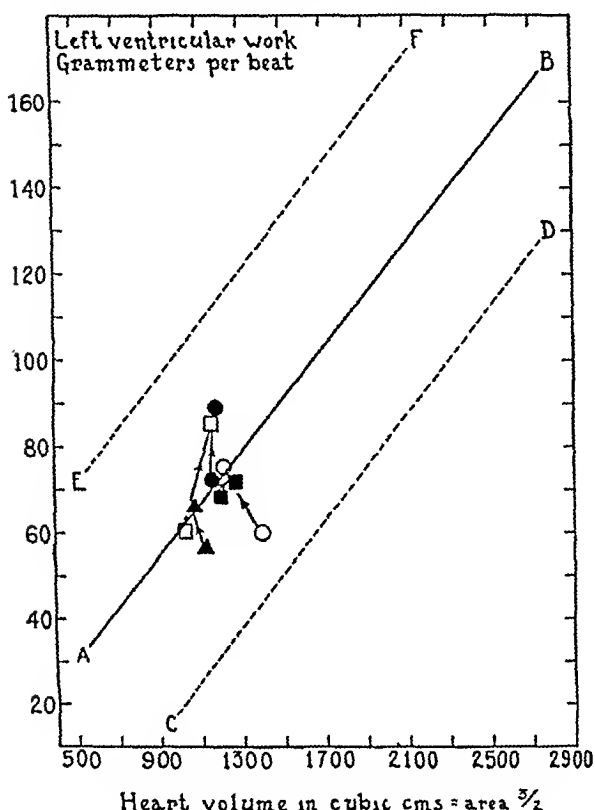


FIG. 8. LEFT VENTRICULAR WORK PER BEAT AND CARDIAC VOLUME.

The data from Table I relating to work of the left ventricle per beat are plotted against the corresponding cardiac volumes. Line *AB* represents the best line, the regression of the work on the area, defined by Starr, Collins and Wood (13, Figure 2) on the basis of a statistical treatment of data from a control group of cases. Lines *CD* and *EF* are placed by these authors at a distance of twice the standard deviation from *AB*. It appears from their observations that a patient falling within zone *CD-EF* has a normal circulatory function, that is to say, the work of the heart is commensurate with its size; on the other hand they found that the values relating to patients who had suffered from cardiac decompensation, fell in a zone below *CD*. The values of the patients suffering from pernicious anemia now being reported fall well within the zone *CD-EF* and, as a matter of fact, lie quite near the best line *AB*. The arrows in each instance point from the first observation during anemia toward the second observation after the hemoglobin had increased. In 4 instances, progression was made toward or even above the best line, and in the fifth remained unchanged.

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#### SUMMARY

1. Measurements of cardiac output, by the acetylene method, of cardiac size, and related circulatory functions, have been made in five cases of pernicious anemia both during the period of anemia, and later during a remission induced by the use of liver extract therapy, when the blood picture was more nearly normal.

2. At low hemoglobin levels in the blood, the

proached more nearly normal values (Figure 7). This result is similar to that growing out of the observations of Tompkins, Brittingham and Drinker (17) who approached this question in another manner. They found that the metabolic rates of patients suffering from pernicious anemia decreased following the transfusion of blood. It appears clear, therefore, both from our own observations and from those already recorded by others, that the oxygen consumption, in short, the basal metabolic rate, may be increased appreciably in the presence of pernicious anemia. A satisfactory explanation for this phenomenon has not yet been advanced. Our data do not permit us to make any positive contribution to this puzzling observation. They indicate, however, as will be shown later, that increased oxygen consumption is not a consequence of increased work of the heart, a notion which has been current up to the present.

That enlargement of the heart in anemic patients occurs as a consequence of both dilatation and hypertrophy is an idea that has been held for many years (18, 19, 7). Most of the conclusions have been based upon clinical and postmortem observations in cases of pernicious anemia. One patient reported by Ball (20) is of especial interest since roentgenological enlargement of the heart was observed during severe secondary anemia, and was followed by decrease in size when the blood count returned to normal. From these observations he concluded that enlargement was due to dilatation rather than hypertrophy. In the case of three patients in the series now being reported, the heart was moderately enlarged or at the upper limits of normal, only two being well within normal limits. That observations made after improvement failed to show significant change in size of the heart may be due in part to the relatively short time covered by observations of each patient.

The cause of cardiac enlargement found in the presence of anemia has been subject to controversy. One notion (11) regards it as a consequence of increased activity of the heart; another (7) attributes it to lack of oxygen in the myocardium. Because of these notions it appeared of interest to ascertain the amount of work done by the heart in patients suffering from pernicious

anemia. Work may be calculated by making use of the formula (21)

$$W = QR + \frac{wV^2}{2g}$$

in which  $W$  equals work done per minute, or per beat;  $Q$  equals volume of blood expelled per minute, or per beat;  $R$  equals mean arterial blood pressure in mm. of Hg  $\times 13.6$ ;  $V$  equals velocity of blood at aorta;  $w$  equals weight of blood;  $g$  equals acceleration due to gravity. The last part of the formula,  $\frac{(wV^2)}{2g}$ , has been omitted in order to make our results comparable to those of Starr et al. (13). By substituting values in this formula, we have calculated the work of the left ventricle both per beat and per minute. Since there is evidence (22) that the work of the right ventricle bears a constant relationship to that of the left, we have concerned ourselves only with the latter. The work per beat done by the left ventricle was found less during the period of increased cardiac output at low hemoglobin levels, than later when the output was lowered and the amount of hemoglobin more nearly normal (Table I).

Taking into account the increased heart rates found in the anemic state, as is done in computing the work per minute, does not reveal increased work in the presence of anemia. This is due for the most part to the considerable increase in the mean arterial pressure which is found at the higher levels of hemoglobin. Since, therefore, the work of the heart appears to be within normal limits there seems to be no point in retaining the notion which is current, that enlargement of the heart in anemia is a consequence of increased cardiac work; on the other hand, by exclusion it lends credence to the view that myocardial anoxemia may be the stimulus which initiates enlargement. Likewise, since we have failed to find the work of the heart increased in these patients, this mechanism does not account for the increased basal metabolic rate.

Stewart and Cohn (23) expressed the opinion that the explanation of the increase in cardiac output with decrease in cardiac size as a consequence of giving digitalis to patients suffering from congestive heart failure, was to be found in Starling's "Law of the Heart" (24). Further-

more, Starr and his associates (12, 13) have presented since then, data indicating that this "Law" applies to basal cardiac work in human beings, as well as to the heart-lung preparation. For it

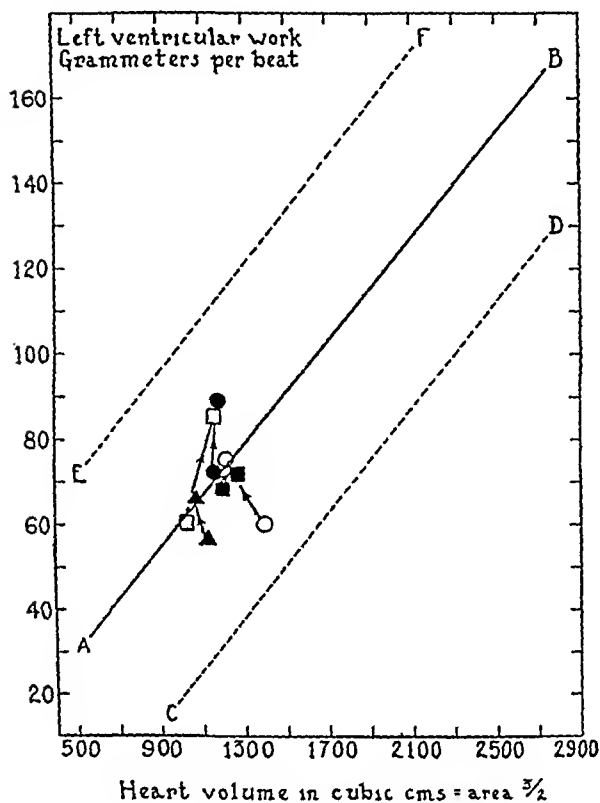


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#### SUMMARY

1. Measurements of cardiac output, by the acetylene method, of cardiac size, and related circulatory functions, have been made in five cases of pernicious anemia both during the period of anemia, and later during a remission induced by the use of liver extract therapy, when the blood picture was more nearly normal.

2. At low hemoglobin levels in the blood, the



stroke-volume and minute-volume output, the cardiac rate, and the oxygen consumption were in all cases elevated, and the arm to tongue circulation time decreased. Changes in the reverse direction occurred as the state of the blood approached normal.

3. In four instances the vital capacities were moderately lowered during anemia.

4. The venous pressure and electrocardiograms showed no significant alterations during the anemic state.

5. There was a rise in both the systolic and diastolic levels of blood pressure in all patients as the hemoglobin concentration increased.

6. Although the cardiac size was moderately increased in three instances, no significant change in size was observed as the hemoglobin approached a normal level.

7. Left ventricular work, both per beat and per minute, was not increased during anemia; in fact, the work per beat was less than when the hemoglobin concentration in the blood was normal. It is suggested that the enlarged hearts found in this type of anemia are due to myocardial anoxemia, rather than to increased cardiac work. The elevated metabolic rates, observed in these patients during the anemic state, were not due to increased cardiac work.

8. The amount of cardiac work done at rest, in anemia, was commensurate with the cardiac size.

9. From these observations it appears that during the anemia of pernicious anemia, the heart is called upon to pump an increased amount of blood per minute, the amount appearing to be a linear function of the hemoglobin concentration. This is accomplished in part by increasing the heart rate, and in part, by maintaining a greater output per beat than they have at higher hemoglobin levels. These alterations are reflected in a shortening of the circulation time; in short, the red blood cells, although fewer in number, move at an increased velocity and, as a consequence, they are used more frequently in their oxygen carrying capacity. Shortening of the circulation time and increase in cardiac output as well as decrease in hemoglobin and increase in oxygen consumption also appear to have linear relationships so that an organ already working at an accelerated rate is

put to the disadvantage of having to maintain a circulation sufficient for the requirements of an increased basal metabolic rate. With increase in the level of hemoglobin, changes in the reverse direction occur. Nevertheless, the hearts of these patients, without congestive heart failure, at rest, mechanically perform the work expected of them for their size.

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# DETERMINATION OF BLOOD ASCORBIC ACID

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(Received for publication December 28, 1936)

Since vitamin C has definitely been identified with ascorbic acid the clinical importance of this substance has increased. However, the interpretation of blood ascorbic acid values has been uncertain, in part, because of the variations in values encountered in routine analyses. Tillmans, Hirsch and Hirsch (1) first developed the method of determining the presence of reduced ascorbic acid in serum. To make it clinically practical, this original method has been modified by Farmer and Abt (2, 3). Their procedure was to use metaphosphoric acid as a deproteinizing agent which had been introduced for this purpose by Fujita and Iwatake (4) and to determine the reduced ascorbic acid in the filtrate with 2:6 dichlorophenol indophenol (5).

Pijoan, Townsend and Wilson (6), in attempting to repeat the experiments of Farmer and Abt (2), noted a considerable loss of ascorbic acid in serum which had been allowed to stand at room or ice-box temperatures for over one hour. The loss of ascorbic acid from the time of withdrawal of the blood from the patient to its immediate analysis in the laboratory is incalculable. This loss is due to the oxidation of ascorbic acid. Barron, de Meio, and Klemperer (7) have pointed out that the oxidation can be achieved by catalysts such as copper or the hemochromogens. Barron, Barron and Klemperer (8) showed that the time required for half-oxidation of ascorbic acid added to serum was reduced by the addition of small amounts of copper. They further demonstrated the presence of a protecting mechanism in the serum against the oxidation of ascorbic acid by copper. This inhibitory substance has been shown by de Caro and Giani (9), Bersin, Köster and Jusatz (10) and Mawson (11) to be glutathione.

By the use of the Warburg apparatus in an atmosphere of 5 per cent  $\text{CO}_2$  and 95 per cent  $\text{O}_2$ , Barron, Barron and Klemperer were able to show that the protection in serum against catalysts was

not complete, the half-oxidation time requiring from 148 to 244 minutes. In ordinary laboratory glassware, using all the required precautions against contamination, and by using Tillmans' method of assay modified by Farmer and Abt, the half oxidation time was found to be about fifty minutes. This would seem to indicate that the ideal time for analysis would be at the time the blood is collected. Even at the pH of the metaphosphoric acid filtrate (pH 2) the oxidation of ascorbic acid by minute traces of copper is pronounced (8). This is shown by the oxidation of ascorbic acid in metaphosphoric acid (Figure 1).

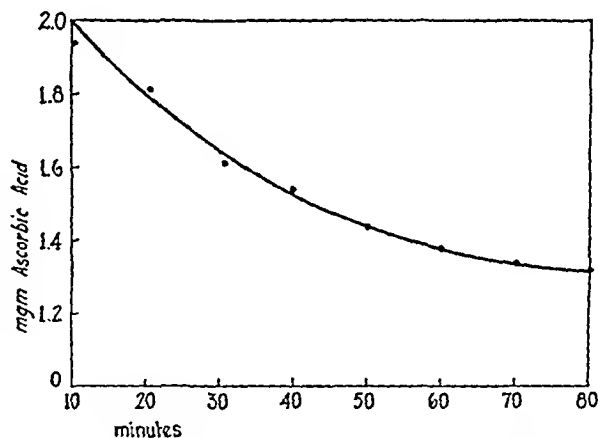


FIG. 1. OXIDATION OF ASCORBIC ACID IN METAPHOSPHORIC ACID FILTRATE. (No KCN.) TEMPERATURE  $25^{\circ}\text{C}$ .

Since KCN had been found to protect the oxidation of ascorbic acid in the serum by added copper and hemochromogens (8), we attempted to investigate its effect on the catalysts ordinarily present in blood. It must be further noted that copper is present in all biological fluids in a concentration sufficient to produce the oxidation of ascorbic acid (12). The following method can be used for the clinical determination of ascorbic acid in serum.

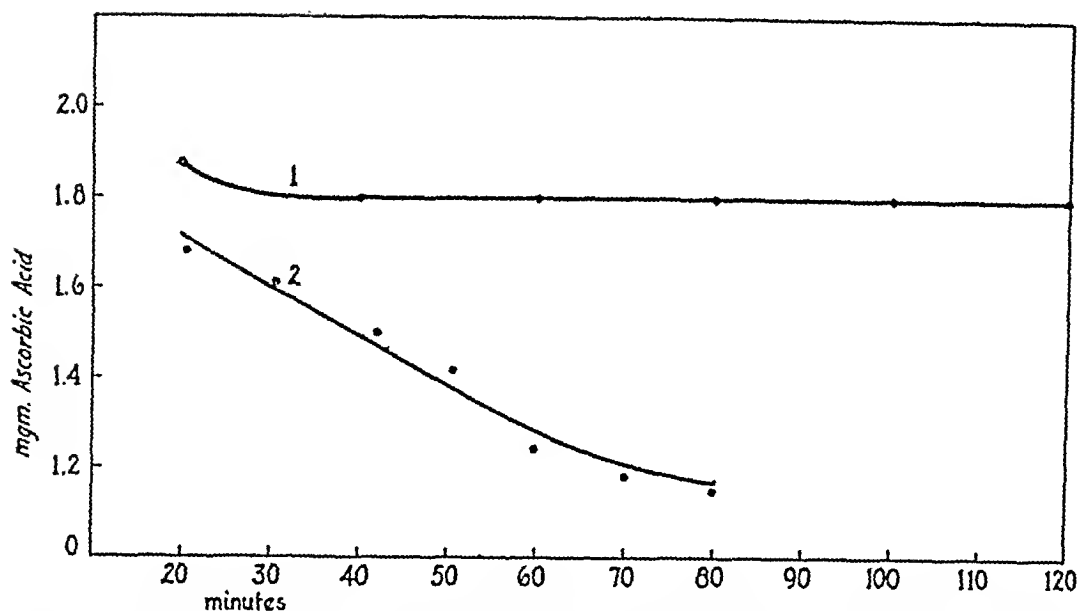


FIG. 2. CURVE 1—INHIBITION OF OXIDATION OF ASCORBIC ACID IN SERUM BY KCN.  
CURVE 2—OXIDATION OF ASCORBIC ACID IN SERUM WITHOUT KCN.

For routine purposes 5 mgm. KCN and 10 mgm. of potassium oxalate are added to test tubes. Blood is collected from a patient's vein, usually fasting, and about 6 to 7 cc. are placed in the tube and centrifuged. Two cubic centimeters of plasma are transferred to a test tube or centrifuge tube. To this is added 2 cc. of distilled water and 6 cc. of 10 per cent metaphosphoric acid (the metaphosphoric acid is made up fresh daily). This mixture is stirred rapidly with a glass rod for thirty seconds and allowed to stand for three minutes. In order to separate the protein precipitate from the protein-free fluid, either filtration through a Whatman 42 filter paper or centrifuging is satisfactory. Two cubic centimeters of either the filtrate or the supernatant fluid is used for the determination. This fluid is placed in a beaker (50 cc.) for titration with 2:6 dichlorophenol indophenol. Titrations to a pink end-point are carried out using a light with a daylight filter. A 1/1000 Molar solution (29 mgm. of dye in 100 cc. of water) is the most efficient for this purpose. In preparing the dye solution it is convenient to dissolve it in water heated to 85° C., shake for fifteen minutes and filter if necessary. The dye is standardized against ascorbic acid (Hoffman-Roche). For purposes of convenience and simplicity the following calculation is included.

$$\frac{\text{cc. of dye used in titration} \times .001 \times 176 \times 100}{0.4} = \frac{\text{cc. dye used} \times 44}{\text{ascorbic acid per 100 cc. plasma.}}$$

0.001 is the concentration of the dye in millimols per cubic centimeter.

176 is the milligrams of ascorbic acid equivalent to 1 millimol.

0.4 is the amount of serum used for the titration.

The whole is multiplied by 100 to give the results in terms of 100 cc. of plasma.

The error in the titration amounts to  $\pm 0.1$  mgm.

The addition of ascorbic acid to serum in which the ascorbic acid value was previously determined yields recovery values as expressed in Table I.

TABLE I  
Recovery of ascorbic acid added to serum containing KCN  
100 milligrams per 100 cubic centimeters of serum

	Originally present	Amount added	Theoretical total	Found after 15 minutes	After ½ hour	After 1 hour
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	.86	5.0	5.86	5.80	5.80	5.80
2	.87	4.0	4.87	4.80	4.80	4.78
3	.86	3.0	3.86	3.84	3.83	3.83
4	.86	2.0	2.86	2.84	2.84	2.84
5	.86	2.0	2.86	2.84	2.82	2.82
6	.86	1.5	2.36	2.30	2.30	2.28
7	.86	1.5	2.36	2.28	2.28	2.28
8	.86	1.0	1.86	1.84	1.82	1.82
9	.86	1.0	1.86	1.84	1.84	1.84

With this method we have obtained values varying from 0.65 to 2.00 mgm. per cent in the blood plasma of 150 normal individuals.

#### CONCLUSION

In assaying ascorbic acid by 2:6 dichlorophenol indophenol there is a considerable loss of ascorbic

acid due to oxidation, which can be prevented by the use of KCN for preservation of the blood.

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# NEUTRALIZATION TESTS IN POLIOMYELITIS. SERA TAKEN DURING THE ACUTE AND CONVALESCENT STAGES OF THE DISEASE AND TESTED WITH A PASSAGE VIRUS AND A STRAIN ISOLATED DURING THE 1935 NEW YORK CITY OUTBREAK<sup>1</sup>

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(Received for publication January 9, 1937)

It has generally been accepted that recovery from paralytic poliomyelitis usually results in the appearance of antibodies in the blood serum (1 to 10). The neutralizing property of human convalescent sera from paralytic patients was first demonstrated in 1910 by Netter and Levaditi (1) and by Flexner and Lewis (2). Since then a number of investigators have confirmed this find-

recorded by several of the early workers. Recently, however, the absence of neutralizing power in convalescent sera has been noted more frequently (16 to 19). The majority of the neutralization tests were performed upon sera obtained from patients years after the onset of the disease; a smaller number were carried out on sera collected within one year; a few were secured

TABLE I  
*Summary of neutralization tests in paralytic cases compiled from the literature*

Investigators	Within one week of onset			Within one year of onset			More than one year after onset		
	Number of cases tested	Number neutralized	Number failed to neutralize	Number of cases tested	Number neutralized	Number failed to neutralize	Number of cases tested	Number neutralized	Number failed to neutralize
Netter and Levaditi (1).....				4	4	0	2	1	1
Peabody, Draper and Dochez (4).....				1	1	0	3	2	1
Anderson and Frost (3).....				1	1	0			
Römer (5).....				2	2	0	1	1	0
Kling and Levaditi (6).....	2	2	0	2	2	0			
Flexner and Amoss (7).....	1	1	0						
Nuzum (8).....				1*	0	1			
Aycock and Kramer (9).....				1	0	1	16	15	1
Fairbrother and Brown (10).....				1	1	0			
Howitt (12).....							20	9	11
Stokes et al. (11).....							2	1	1
Harmon and Harkins (15).....	3	1	2	5	4	1			
Shaughnessy, Harmon and Gordon (16).....				4	2	2	10	7	3
Schultz and Gebhardt (17).....							4	2	2
Jungeblut and Smith (18).....							26	15	11
Paul and Trask (19).....				7	1	6			
Total.....	6	4	2	29	18	11	84	53	31

\* Recently recovered.

ing (Table I). This tabulation does not include tests performed on pooled convalescent sera or on sera of patients who had received serum therapy (11 to 15). An occasional failure of convalescent serum to neutralize poliomyelitis virus was

during the first week of illness. The proportion of patients showing neutralizing substances was approximately the same, regardless of the time after the onset of the disease that the sera were collected (Table I). This would suggest that the neutralizing substances either developed shortly after the onset of the disease or were present at the time of the infection. The former idea was

<sup>1</sup> This work was made possible by grants from the Rockefeller Foundation and the President's Birthday Commission for Infantile Paralysis Research.





# NEUTRALIZATION TESTS IN POLIOMYELITIS. SERA TAKEN DURING THE ACUTE AND CONVALESCENT STAGES OF THE DISEASE AND TESTED WITH A PASSAGE VIRUS AND A STRAIN ISOLATED DURING THE 1935 NEW YORK CITY OUTBREAK<sup>1</sup>

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TABLE I  
Summary of neutralization tests in paralytic cases compiled from the literature

Investigators	Within one week of onset			Within one year of onset			More than one year after onset		
	Number of cases tested	Number neutralized	Number failed to neutralize	Number of cases tested	Number neutralized	Number failed to neutralize	Number of cases tested	Number neutralized	Number failed to neutralize
Netter and Levaditi (1).....				4	4	0	2	1	1
Peabody, Draper and Dochez (4).....				1	1	0	3	2	1
Anderson and Frost (3).....				1	1	0			
Römer (5).....				2	2	0	1	1	0
Kling and Levaditi (6).....	2	2	0	2	2	0			
Flexner and Amoss (7).....	1	1	0						
Nuzum (8).....				1*	0	1			
Aycock and Kramer (9).....				1	0	1	16	15	1
Fairbrother and Brown (10).....				1	1	0			
Howitt (12).....							20	9	11
Stokes et al. (11).....							2	1	1
Harmon and Harkins (15).....	3	1	2	5	4	1			
Shaughnessy, Harmon and Gordon (16).....				4	2	2	10	7	3
Schultz and Gebhardt (17).....							4	2	2
Jungeblut and Smith (18).....							26	15	11
Paul and Trask (19).....				7	1	6			
Total.....	6	4	2	29	18	11	84	53	31

\* Recently recovered.

ing (Table I). This tabulation does not include tests performed on pooled convalescent sera or on sera of patients who had received serum therapy (11 to 15). An occasional failure of convalescent serum to neutralize poliomyelitis virus was

during the first week of illness. The proportion of patients showing neutralizing substances was approximately the same, regardless of the time after the onset of the disease that the sera were collected (Table I). This would suggest that the neutralizing substances either developed shortly after the onset of the disease or were present at the time of the infection. The former idea was

<sup>1</sup>This work was made possible by grants from the Rockefeller Foundation and the President's Birthday Commission for Infantile Paralysis Research.



suspended in an equal amount of glycerine (Kahlbaum) by weight, and kept frozen. Small portions were removed, ground with sand, and to each gram was added 10 cc. of distilled water to make a 10 per cent suspension. The suspension was centrifuged lightly, and the resulting supernatant was referred to as a 5 per cent cord suspension because half of the gram of material mixed with the water was glycerine. Five different batches of infectious cords, each consisting of 4 or 5 spinal cords obtained from monkeys at the height of paralysis, were used. Four of the batches were used from July to November 1935, and the fifth from then until the completion of the work in October 1936. The virus, stored in this way, maintained its potency at a fairly uniform level of infectivity, as indicated by the results of repeated tests carried out with a batch over a period of 12 months.

The infectivity of each batch of virus was determined by making serial dilutions of the 5 per cent suspension with distilled water. This was accomplished by the addition of 0.5 to 1.0 cc. of virus suspension to the required amount of diluent, and then after being shaken for several minutes, 0.13 cc. was measured off with a 0.2 cc. pipette. Usually, 0.13 cc. of a 5 per cent suspension diluted 40 to 100 times and added to 0.6 cc. of saline or normal monkey serum resulted in infection.

*Recently isolated strain.* The virus was obtained from the nasal secretions of an acute bulbar case of poliomyelitis on the 9th day of illness. The washings were passed through a Seitz filter and concentrated *in vacuo* to approximately 5 cc. This was then inoculated intracerebrally and intraperitoneally into a monkey. For the tests described in this paper a single cord from the second passage was used, being kept in glycerine at freezing temperature. In making up the virus suspension, small pieces from 6 to 8 segments were used as previously described (23). The infectivity of the cord was sufficient to produce a rapid and complete paralysis in the usual incubation period when 0.13 cc. of a 5 per cent suspension diluted 20 to 40 times was injected. The virulence of this strain was, therefore, comparable to that of the passage virus.

*Neutralization test.* The neutralization or protection test was carried out as follows: 0.13 cc. of a 1:10 or 1:2 dilution of 5 per cent virus was added to 0.6 cc. of serum; the proportion was 0.1 cc. of diluted virus suspension to 0.45 cc. of serum. The mixtures were incubated for 2 hours at 37° C. and then kept in the ice box for approximately 2 hours. Of this mixture 0.5 to 0.6 cc. was inoculated into the frontal lobe of a monkey. A positive control, consisting of human serum known to have protective substances, was used in each experiment. Likewise, a negative control, consisting of normal monkey serum without protective properties, was included. When the serum failed to protect, the animal became paralyzed. With few exceptions, once paralysis set in, the animals became prostrated. When the paralysis was definite but failed to involve all extremities, it was referred to as "incomplete paralysis."

The usual minimal dose which was used to test the sera in this work was 0.13 cc. of a 1:10 dilution of 5 per

cent virus suspension. If the serum protected, a 1:2 dilution of the virus suspension was used in the next test. Protective substances were considered present only when the animal survived the greater dose. A more concentrated virus suspension was used to offset the irregularities which can occur in carrying out tests with small amounts of virus. When irregularities occurred, several retests were made and the average result recorded. Some of the sera were tested with as many as 3 different batches of virus with fairly consistent results.

Macacus rhesus monkeys weighing 2½ to 4 kilos were used. Those surviving were not used again within the month. After an animal was used 3 times, it was injected with 0.13 cc. of a 1:10 or 1:2 dilution of a 5 per cent virus suspension added to 0.6 cc. of saline or normal monkey serum to determine whether the protection tests carried out upon the monkey were valid. This was necessary because an occasional monkey is naturally resistant. Daily temperatures were taken for at least two weeks after inoculation.

#### EXPERIMENTAL

The sera of 82 paralytic, 32 non-paralytic and 3 encephalitic cases were tested in the acute stage of the disease, that is, within a week after the onset. Many of the sera were retested at intervals during convalescence. Thirty-three experiments were carried out. The following 9 were selected as typical protocols.

#### *Neutralization tests with sera obtained in the acute stages of the disease*

*Experiment 1.* The sera of 16 paralytic and 13 non-paralytic patients ranging from 5 months to 28 years of age were tested. It was found (Table II) that the sera of 6 of 7 paralytics 5 years and under and 2 non-paralytics in the same age group, failed to neutralize a 1:10 dilution of a 5 per cent virus suspension. In the group over 5 years the sera of 8 of 9 paralytics failed to protect; all these were tested with a 1:2 dilution of virus suspension. On the other hand, the sera of 9 of 11 non-paralytics over 5 years of age neutralized either a 1:10 or a 1:2 dilution; seven of them were tested against the 1:2 dilution of virus.

*Experiment 2.* Certain sera of the first and other experiments which had given protection with a given test dose of virus, were retested with larger doses. Sera that previously had neutralized 0.13 cc. of a 1:10 dilution were retested with a 1:2 dilution of a 5 per cent virus suspension; and those that had neutralized the latter amount

supported by Kling and Levaditi (6) who found neutralizing substances three and five days after the onset of the disease, and by Flexner and Amoss (7) who reported a similar finding at six days. The latter possibility was favored by Harmon and coworkers (14) who found neutralizing substances present in the serum of a patient before the onset of paralysis.

The higher incidence of neutralizing substances found in the convalescent sera by the early workers might have been due to chance in dealing with small numbers or to differences in technique of performing the tests. The age of the patient and the virulence of the virus must also be considered.

Very few studies have been carried out on sera from non-paralytic patients. Peabody, Draper and Dochez (4) found no neutralizing power in the serum of a child 7 days after the onset of the disease, while that of another child neutralized at 21 days. Paul and Trask (19) reported the case of a 6 year old non-paralytic child whose serum failed to neutralize both in the acute stage and 1 year later. Harmon and Harkins (15) demonstrated the presence of neutralizing substances in the serum from an 11 year old child obtained 5 days and again 5 months after the onset of the disease. Neutralization tests have also been recorded by several other investigators (1, 3, 5, 6) upon blood from patients about whom insufficient clinical data were given to determine whether they could be classified as non-paralytic or abortive cases of poliomyelitis.

Neutralizing substances have frequently been reported in the sera of so-called normal individuals (9, 16, 20). The incidence of these antibodies appears to increase with age (9, 16). Various workers (9, 16, 20) have found that the sera of at least 50 per cent of normal adults have neutralizing power. Paul and Trask (21) in a review of reports on neutralization tests carried out since 1929, pointed out that the neutralizing power of normal sera exceeded that of convalescent sera in all age groups.

All of the above recorded tests were performed with a monkey passage virus. In addition, Paul and Trask (19) tested 7 sera with a recently isolated human strain of virus, as well as with a passage strain. They found that neutralizing substances were present in 6 of the 7 sera tested

with the former strain as compared to one with the latter. Likewise, Howitt (22) found that 7 convalescent sera neutralized a recently isolated strain, while only 4 of them neutralized a passage virus.

In spite of so much contradictory evidence, the idea still prevails that recovery from an attack of poliomyelitis results in the development of antibodies. It, therefore, seemed advisable to investigate the matter further by tests on a sufficiently large number of sera taken at frequent intervals following the onset of the disease.

The purpose of this work was: (1) To test for the presence or absence of neutralizing substances in the sera of both paralytic and non-paralytic individuals of different ages in the acute and convalescent stages of the disease; (2) To determine whether poliomyelitis can develop in the presence of protective substances; (3) To determine whether paralytic or non-paralytic cases with no demonstrable protective substances in the acute stage of the disease developed them within 12 to 16 months after the onset; (4) To carry out the above tests with the F1 passage virus and with a strain of virus isolated during the summer of 1935.

#### METHODS

Sera were obtained from patients who were admitted to the Willard Parker Hospital for poliomyelitis during the summer and fall of 1935, when over 2,000 cases were reported in New York City. In each instance the diagnosis was established by clinical findings and examination of spinal fluids. Only those patients who had a definite loss of muscle function were classified as paralytic. Non-paralytic cases were those who had an acute onset with meningeal involvement and pleocytosis of the spinal fluid. Every case was seen by one of us and a careful follow-up of the non-paralytic and the paralytic patients was carried out. In this way, it was possible to eliminate from the non-paralytic group those patients who subsequently developed muscle weakness or paralysis. We also correlated, in the paralytics, the degree of muscle recovery with the results of the neutralization tests.

The work was begun in July 1935, and the patients were bled at short intervals during the following 9 months. A number were also bled 12 to 16 months after the onset. The majority of the sera were tested within a few weeks after collection. When comparative tests between early and later bleedings were made, the sera, which had been stored for 6 to 7 months, were again tested. All specimens were cultured before use and were kept at 2° to 4° C.

*Passage virus and preparation of suspension.* The passage virus (F1 strain) was ground without abrasive,

suspended in an equal amount of glycerine (Kahlbaum) by weight, and kept frozen. Small portions were removed, ground with sand, and to each gram was added 10 cc. of distilled water to make a 10 per cent suspension. The suspension was centrifuged lightly, and the resulting supernatant was referred to as a 5 per cent cord suspension because half of the gram of material mixed with the water was glycerine. Five different batches of infectious cords, each consisting of 4 or 5 spinal cords obtained from monkeys at the height of paralysis, were used. Four of the batches were used from July to November 1935, and the fifth from then until the completion of the work in October 1936. The virus, stored in this way, maintained its potency at a fairly uniform level of infectivity, as indicated by the results of repeated tests carried out with a batch over a period of 12 months.

The infectivity of each batch of virus was determined by making serial dilutions of the 5 per cent suspension with distilled water. This was accomplished by the addition of 0.5 to 1.0 cc. of virus suspension to the required amount of diluent, and then after being shaken for several minutes, 0.13 cc. was measured off with a 0.2 cc. pipette. Usually, 0.13 cc. of a 5 per cent suspension diluted 40 to 80 times and added to 0.6 cc. of saline or normal monkey serum resulted in infection.

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cent virus suspension. If the serum protected, a 1:2 dilution of the virus suspension was used in the next test. Protective substances were considered present only when the animal survived the greater dose. A more concentrated virus suspension was used to offset the irregularities which can occur in carrying out tests with small amounts of virus. When irregularities occurred, several retests were made and the average result recorded. Some of the sera were tested with as many as 3 different batches of virus with fairly consistent results.

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#### EXPERIMENTAL

The sera of 82 paralytic, 32 non-paralytic and 3 encephalitic cases were tested in the acute stage of the disease, that is, within a week after the onset. Many of the sera were retested at intervals during convalescence. Thirty-three experiments were carried out. The following 9 were selected as typical protocols.

#### *Neutralization tests with sera obtained in the acute stages of the disease*

*Experiment 1.* The sera of 16 paralytic and 13 non-paralytic patients ranging from 5 months to 28 years of age were tested. It was found (Table II) that the sera of 6 of 7 paralytics 5 years and under and 2 non-paralytics in the same age group, failed to neutralize a 1:10 dilution of a 5 per cent virus suspension. In the group over 5 years the sera of 8 of 9 paralytics failed to protect; all these were tested with a 1:2 dilution of virus suspension. On the other hand, the sera of 9 of 11 non-paralytics over 5 years of age neutralized either a 1:10 or a 1:2 dilution; seven of them were tested against the 1:2 dilution of virus.

*Experiment 2.* Certain sera of the first and other experiments which had given protection with a given test dose of virus, were retested with larger doses. Sera that previously had neutralized 0.13 cc. of a 1:10 dilution were retested with a 1:2 dilution of a 5 per cent virus suspension; and those that had neutralized the latter amount

TABLE II

*Summary of neutralization tests with sera taken in the first week of the disease*

Patient's initials	Age	Type of case	Time after onset	Dilution of 5 per cent virus	Amount of virus	Amount of serum	Number of monkey	Results
			days		cc.	cc.		
Negative control.....	Normal monkey serum			1 : 10	0.13	0.60	J 127	Paralyzed 7 days
Negative control.....	Normal monkey serum			1 : 2	0.13	0.60	J 35	Paralyzed 7 days
Positive control.....	Normal adult human serum (M.B.)			*	0.20	0.45	J 1219	No paralysis
Positive control.....	Normal adult human serum (M.B.)			*	0.45	0.45	617	Paralyzed 11 days
S. S.....	5 mos.	Paralytic	7	1 : 10	0.13	0.60	J 92	Paralyzed 10 days
D. O'B.....	16 mos.	Paralytic	3	1 : 10	0.13	0.60	J 107	Paralyzed 6 days
	years							
H. D.....	2	Paralytic	4	1 : 10	0.13	0.60	J 124	No paralysis
R. M.....	4	Paralytic	5	1 : 10	0.13	0.60	J 108	Paralyzed 9 days
V. M.....	4	Paralytic	3	1 : 10	0.13	0.60	J 102	Paralyzed 9 days
G. C.....	5	Paralytic	5	1 : 10	0.13	0.60	J 112	Paralyzed 6 days
P. B.....	5	Paralytic	3	1 : 10	0.13	0.60	J 111	Paralyzed 14 days
C. M.....	6	Paralytic	5	1 : 2	0.13	0.60	J 122	Paralyzed 8 days
R. A.....	7	Paralytic	2	1 : 10	0.13	0.60	J 120	No paralysis
R. A.....	7	Paralytic	2	1 : 2	0.13	0.60	J 119	No paralysis
J. B.....	8	Paralytic	1	1 : 2	0.13	0.60	J 105	Paralyzed 7 days
M. G.....	8	Paralytic	3	1 : 10	0.13	0.60	J 121	Paralyzed 15 days
M. G.....	8	Paralytic	3	1 : 2	0.13	0.60	J 53	Paralyzed 8 days
M. R.....	9	Paralytic	4	1 : 2	0.13	0.60	J 103	Paralyzed 16 days
T. McT.....	15	Paralytic	6	1 : 2	0.13	0.60	J 90	Paralyzed 6 days
J. M.....	16	Paralytic	4	1 : 2	0.13	0.60	J 91	Paralyzed 6 days
E. P.....	21	Paralytic	4	1 : 2	0.13	0.60	J 97	Paralyzed 6 days
V. C.....	28	Paralytic	1	1 : 2	0.13	0.60	J 93	Paralyzed 6 days
P. M.....	3	Non-paralytic	7	1 : 10	0.13	0.60	J 100	Paralyzed 7 days
H. B.....	5	Non-paralytic	3	1 : 10	0.13	0.60	J 113	Paralyzed 4 days
C. E.....	6	Non-paralytic	3	1 : 10	0.13	0.60	J 114	No paralysis
L. W.....	8	Non-paralytic	2	1 : 10	0.13	0.60	J 123	No paralysis
L. W.....	8	Non-paralytic	2	1 : 2	0.13	0.60	J 70	No paralysis
M. E.....	9	Non-paralytic	3	1 : 2	0.13	0.60	J 96	No paralysis
H. E.....	10	Non-paralytic	3	1 : 10	0.13	0.60	J 115	No paralysis
J. B.....	13	Non-paralytic	1	1 : 2	0.13	0.60	J 98	No paralysis
C. S.....	15	Non-paralytic	2	1 : 2	0.13	0.60	J 99	Paralyzed 9 days
A. P.....	16	Non-paralytic	2	1 : 2	0.13	0.60	J 95	No paralysis
R. J.....	16	Non-paralytic	4	1 : 2	0.13	0.60	J 106	No paralysis
A. C.....	16	Non-paralytic	6	1 : 2	0.13	0.60	J 128	Paralyzed 16 days
B. M.....	22	Non-paralytic	2	1 : 2	0.13	0.60	J 104	No paralysis
M. W.....	27	Non-paralytic	4	1 : 2	0.13	0.60	J 94	No paralysis

\* Undiluted.

were tested with 0.13 cc. or more of a 5 per cent suspension. The results (Table III) showed that the sera of 5 of 8 non-paralytcs and two paralytcs protected when the larger quantity of virus was used.

*Summary of results of all tests on sera collected in the acute stage of the disease*

The results of all of the first-week bleedings (Table IV) indicated that protective substances were present in 14 of 82 paralytcs during the acute stage of the disease. In the non-paralytic group, on the other hand, they were present in

18 of 32 sera. When protective substances were present, they were detected as early as 1 and 2 days after the onset of the disease both in paralytic and non-paralytic cases. Four of the 82 patients were tested in the preparalytic stage. Two of them were found to have protective substances.

*Protective substances in normal urban individuals*

It was decided to test sera of a group of so-called normal residents of New York City between 11 and 25 years of age to determine whether the virus and technique used in the

TABLE III

*Neutralization tests in the first week of the disease. (These sera had previously neutralized a smaller dose of virus)*

Patient's initials	Age	Type of case	Time after onset	Dilution of 5 per cent virus	Amount of virus	Amount of serum	Number of monkey	Results
	years		days		cc.	cc.		
Negative control.....	Normal monkey serum			1 : 10	0.13	0.60	J 207	Paralyzed 7 days
Negative control.....	Normal monkey serum			1 : 2	0.13	0.60	J 126	Paralyzed 6 days
Positive control.....	Normal adult human serum (M.B.)			*	0.20	0.45	J 208	Partial paralysis
Positive control.....	Normal adult human serum (M.B.)			1 : 2	0.13	0.60	J 215	No paralysis
R. G.....	3	Non-paralytic	4	*	0.20	0.45	J 239	No paralysis
C. E.....	6	Non-paralytic	3	1 : 2	0.13	0.45	J 234	Paralyzed 15 days
L. W.....	8	Non-paralytic	2	*	0.20	0.45	J	No paralysis
L. W.....	8	Non-paralytic	2	*	0.45	0.45	J 229	No paralysis
J. B.....	13	Non-paralytic	1	*	0.20	0.45	N 17	No paralysis
R. J.....	16	Non-paralytic	4	*	0.20	0.45	N 19	Paralyzed 7 days
A. P.....	16	Non-paralytic	2	*	0.20	0.45	J 119	No paralysis
A. P.....	16	Non-paralytic	2	*	0.45	0.45	J 211	No paralysis
B. M.....	22	Non-paralytic	2	*	0.20	0.45	J 237	No paralysis
M. W.....	27	Non-paralytic	4	*	0.20	0.45	N 8	Paralyzed 9 days
H. D.....	2	Paralytic	5	1 : 2	0.13	0.60	J 220	No paralysis
L. E.....	8	Paralytic	3	1 : 2	0.13	0.60	J 216	No paralysis

\* Undiluted.

experiments produced approximately the same proportion of neutralizations reported by others (9, 16, 21). Moreover, this would enable us also to compare the results of these tests with those of paralytic and non-paralytic patients in the acute stage of the disease.

TABLE IV

*Summary of neutralization tests with sera taken in the first week of the disease*

Age group	Paralytics			Non-paralytics			Encephalitic		
	Number tested	Sera neutralized	Sera failed to neutralize	Number tested	Sera neutralized	Sera failed to neutralize	Number tested	Sera neutralized	Sera failed to neutralize
years									
1-5....	26	2	24	6	1	5			
6-10...	25	4	21	14	10	4	3	1	2
11-17..	19	4	15	10	5	5			
Adults.	12	4	8	2	2	0			
Totals..	82	14	68	32	18	14	3	1	2

*Experiment 3.* The sera of 18 individuals from 11 to 25 years of age were tested by the use of 0.6 cc. of serum mixed with 0.13 cc. of a 2½ per cent (a 1:2 dilution of 5 per cent) virus suspension. Protection occurred with 9 of the 18 sera. The proportion of positive neutralizations in the group approximates that found by

other workers for normal urban adolescents and adults. Protective substances were found in only 8 of the 31 sera from paralytic patients over 10 years of age during the acute stage (Table IV). In the non-paralytic patients of the same age group, 7 of the 12 sera protected. Thus, the incidence of protective substances during the acute stage of the disease in the paralytic group was lower than that in the normal urban residents, while in the non-paralytic group it was approximately the same.

#### *Protection tests with sera secured during the first 9 months of convalescence*

Repeated tests were carried out upon the sera of 44 paralytic, 13 non-paralytic and 2 encephalitic patients at various intervals from two weeks to nine months after the onset of the disease. The sera of a few of these individuals had protected in the first week of their illness, but the majority had failed to do so.

#### *Protection tests with convalescent sera from individuals whose sera failed to protect in the first week of the disease*

The following three experiments are typical of the results obtained.



*Experiment 4.* The sera of 2 paralytic cases obtained 6 weeks and 4 months, respectively, after the onset were tested together with those obtained in the acute stage of the disease. Similar tests were carried out with specimens of serum from a non-paralytic case and also with specimens obtained from an encephalitic case 3

child, G. C., specimens obtained in the 1st, 3d, and 12th weeks were tested against graded dilutions of virus (1:10 to 1:80). At the same time specimens from 15 paralytics and non-paralytics obtained 1 to 8 months after the onset were tested in the usual way against 1:10 and 1:2 dilutions of 5 per cent virus. From 3 of

TABLE V

*Neutralization tests with convalescent sera of individuals whose sera failed to neutralize in the first week*

Patient's initials	Age	Type of case	Time after onset	Dilution of 5 per cent virus	Amount of virus	Amount of serum	Number of monkey	Results
	years				cc.	cc.		
Negative control.....		Normal monkey serum		1 : 10	0.13	0.60	J 75	Paralyzed 8 days
Negative control.....		Normal monkey serum		1 : 5	0.13	0.60	J 63	Paralyzed 6 days
Negative control.....		Normal monkey serum		1 : 2	0.13	0.60	J 66	Paralyzed 8 days
Positive control.....		Normal adult human serum (M.B.)		1 : 10	0.13	0.60	J 64	No paralysis
Positive control.....		Normal adult human serum (M.B.)		1 : 5	0.13	0.60	J 74	No paralysis
Positive control.....		Normal adult human serum (M.B.)		1 : 2	0.13	0.60	145	No paralysis
G. M.....	13	Paralytic	2 days	1 : 10	0.13	0.60	J 79	Paralyzed 6 days
G. M.....	13	Paralytic	2 days	1 : 5	0.13	0.60	J 88	Prostrate 6 days
G. M.....	13	Paralytic	2 days	1 : 2	0.13	0.60	226	Paralyzed 8 days
G. M.....	13	Paralytic	4 months	1 : 10	0.13	0.60	J 87	Paralyzed 7 days
G. M.....	13	Paralytic	4 months	1 : 5	0.13	0.60	J 84	Paralyzed 7 days
G. M.....	13	Paralytic	4 months	1 : 2	0.13	0.60	J 56	Paralyzed 7 days
R. H.....	6	Paralytic	2 days	1 : 10	0.13	0.60	J 83	Paralyzed 9 days
R. H.....	6	Paralytic	2 days	1 : 5	0.13	0.60	J 77	Paralyzed 5 days
R. H.....	6	Paralytic	2 days	1 : 2	0.13	0.60	J 28	Paralyzed 7 days
R. H.....	6	Paralytic	6 weeks	1 : 10	0.13	0.60	J 85	Paralyzed 6 days
R. H.....	6	Paralytic	6 weeks	1 : 5	0.13	0.60	J 81	Paralyzed 9 days
R. H.....	6	Paralytic	6 weeks	1 : 2	0.13	0.60	J 1	Paralyzed 9 days
T. S.....	6	Non-paralytic	3 days	1 : 10	0.13	0.60	J 80	Paralyzed 6 days
T. S.....	6	Non-paralytic	3 days	1 : 5	0.13	0.60	J 76	Paralyzed 8 days
T. S.....	6	Non-paralytic	3 days	1 : 2	0.13	0.60	J 57	Paralyzed 6 days
T. S.....	6	Non-paralytic	3 weeks	1 : 10	0.13	0.60	J 69	Paralyzed 12 days
T. S.....	6	Non-paralytic	3 weeks	1 : 5	0.13	0.60	J 78	Paralyzed 7 days
T. S.....	6	Non-paralytic	3 weeks	1 : 2	0.13	0.60	211	Paralyzed 6 days
M. F.....	7	Encephalitic	19 days	1 : 10	0.13	0.60	J 65	Paralyzed 7 days
M. F.....	7	Encephalitic	19 days	1 : 5	0.13	0.60	J 67	Paralyzed 12 days
M. F.....	7	Encephalitic	19 days	1 : 2	0.13	0.60	J 32	Paralyzed 7 days
M. F.....	7	Encephalitic	5 months	1 : 10	0.13	0.60	J 89	Prostrate 6 days
M. F.....	7	Encephalitic	5 months	1 : 5	0.13	0.60	J 86	Paralyzed 8 days
M. F.....	7	Encephalitic	5 months	1 : 2	0.13	0.60	J 18	Paralyzed 6 days

weeks and 5 months, respectively, after the onset. Each serum was tested with three different dilutions of virus. The results of these tests (Table V) failed to show any evidence that protective substances had developed in these four convalescent sera.

*Experiment 5.* To determine whether any evidence whatsoever of protective power could be detected in convalescent sera from a paralytic

these individuals, the specimens collected during the first week and 3 subsequent specimens were tested. The results (Table VI) of this experiment agreed with those of the previous one, in that none of the 12 sera from paralytics had shown protective power when tested from 2 to 8 months after the onset. Likewise, the 3 sera from non-paralytics failed to neutralize the virus.

Unfortunately, the positive control serum failed

TABLE VI

*Neutralization tests with convalescent sera from individuals whose sera failed to neutralize in the first week*

Patient's initials	Age	Type of case	Time after onset	Dilution of 5 per cent virus	Amount of virus	Amount of serum	Number of monkey	Results
	years				cc.	cc.		
Negative control		Normal monkey serum		1 : 2	0.13	0.60	J 197	Paralyzed 6 days
Negative control		Normal monkey serum		1 : 10	0.13	0.60	J 199	Paralyzed 8 days
Negative control		Normal monkey serum		1 : 20	0.13	0.60	J 198	Paralyzed 6 days
Negative control		Normal monkey serum		1 : 40	0.13	0.60	J 196	Paralyzed 7 days
Negative control		Normal monkey serum		1 : 80	0.13	0.60	J 194	Paralyzed 6 days
Positive control		Normal adult human serum (M.B.)		*	0.20	0.45	J 178	Paralyzed 15 days
Positive control		Normal adult human serum (M.B.)		*	0.45	0.45	J 64	Partial paralysis 15 days
Positive control		Normal adult human serum (M.B.)		*	0.45	0.45†	J 74	Paralyzed 10 days
G. C.	5	Paralytic	5 days	1 : 10	0.13	0.60	J 186	Paralyzed 7 days
G. C.	5	Paralytic	5 days	1 : 20	0.13	0.60	J 187	Paralyzed 7 days
G. C.	5	Paralytic	5 days	1 : 80	0.13	0.60	J 185	Paralyzed 9 days
G. C.	5	Paralytic	19 days	1 : 10	0.13	0.60	J 191	Paralyzed 7 days
G. C.	5	Paralytic	19 days	1 : 20	0.13	0.60	J 190	Paralyzed 36 days
G. C.	5	Paralytic	19 days	1 : 40	0.13	0.60	J 189	Paralyzed 9 days
G. C.	5	Paralytic	19 days	1 : 80	0.13	0.60	J 188	Paralyzed 8 days
G. C.	5	Paralytic	3 months	1 : 10	0.13	0.60	J 192	Paralyzed 7 days
G. C.	5	Paralytic	3 months	1 : 20	0.13	0.60	J 193	Paralyzed 9 days
G. C.	5	Paralytic	3 months	1 : 40	0.13	0.60	J 175	Paralyzed 14 days
G. C.	5	Paralytic	3 months	1 : 80	0.13	0.60	B.U.	Died 8th day—Intercurrent infection
R. M.	4	Paralytic	15 days	1 : 10	0.13	0.60	J 170	Paralyzed 9 days
R. M.	4	Paralytic	26 days	1 : 10	0.13	0.60	J 164	Paralyzed 7 days
R. M.	4	Paralytic	3½ months	1 : 10	0.13	0.60	J 163	Paralyzed 4 days
V. M.†	4	Paralytic	2 days	1 : 10	0.13	0.60	J 173	Paralyzed 7 days
V. M.	4	Paralytic	19 days	1 : 10	0.13	0.60	J 174	Paralyzed 7 days
V. M.	4	Paralytic	4 months	1 : 10	0.13	0.60	J 168	Paralyzed 6 days
V. M.	4	Paralytic	7 months	1 : 10	0.13	0.60	J 167	Paralyzed 6 days
J. S.	3	Paralytic	8 months	1 : 10	0.13	0.60	J 171	Paralyzed 7 days
J. C.	8	Paralytic	8 months	1 : 10	0.13	0.60	J 172	Paralyzed 7 days
E. S.	5	Paralytic	2 months	1 : 2	0.13	0.60	N 12	Paralyzed 11 days
E. S.	5	Paralytic	6 months	1 : 2	0.13	0.60	J 158	Paralyzed 7 days
D. F.	17	Paralytic	7 weeks	1 : 2	0.13	0.60	J 182	Paralyzed 6 days
D. F.	17	Paralytic	7½ months	1 : 2	0.13	0.60	J 2	Paralyzed 9 days
K. G.	31	Paralytic	3 months	1 : 2	0.13	0.60	J 156	Paralyzed 8 days
K. G.	31	Paralytic	3 months	*	0.10	0.60	R 68	Paralyzed 6 days
K. G.	31	Paralytic	8 months	1 : 2	0.13	0.60	J 155	Paralyzed 7 days
K. G.	31	Paralytic	8 months	*	0.10	0.60	135	Paralyzed 11 days
S. H.	3	Paralytic	6 months	1 : 10	0.13	0.60	J 160	Paralyzed 8 days
S. H.	3	Paralytic	8½ months	1 : 2	0.13	0.60	R 49	Paralyzed 8 days
C. H.	6	Paralytic	1 month	1 : 2	0.13	0.60	49§	No paralysis
C. H.	6	Paralytic	7 months	1 : 2	0.13	0.60	J 159	Paralyzed 15 days
A. G.	29	Paralytic	3 weeks	1 : 2	0.13	0.60	R 62	Paralyzed 8 days
A. G.	29	Paralytic	7 months	1 : 2	0.13	0.60	J 157	Paralyzed 5 days
S. T.	8	Paralytic	7 months	1 : 2	0.13	0.60	J 176	Paralyzed 9 days
J. S.	5	Non-paralytic	6 months	1 : 10	0.13	0.60	J 165§	No paralysis
W. S.	11	Non-paralytic	6 months	1 : 10	0.13	0.60	J 166	Paralyzed 6 days
K. S.	11	Non-paralytic	7 months	1 : 10	0.13	0.60	J 177	Paralyzed 15 days
P. M.	3	Non-paralytic	3 weeks	1 : 10	0.13	0.60	J 180	Paralyzed 7 days
P. M.	3	Non-paralytic	3 months	1 : 10	0.13	0.60	J 181	Paralyzed 7 days
P. M.	3	Non-paralytic	6 months	1 : 10	0.13	0.60	J 183	Paralyzed 7 days

\* Undiluted.

† Bulbar case.

‡ 1 : 6 dilution of serum.

§ These animals were subsequently proven to be resistant to poliomyelitis virus. The results have been omitted in the tabulations.

to neutralize 0.2 cc. of a 5 per cent virus suspension, an amount of the same batch of virus that this serum had neutralized in Experiment 1; the injected animal contracted poliomyelitis after a prolonged incubation period. In several other experiments, this serum had neutralized 0.13 cc. of a 1:2 dilution of a 5 per cent suspension, the usual test dose. In the next experiment, the same serum neutralized a similar test dose of the same batch of virus.

*Experiment 6.* Inasmuch as the positive control serum in Experiment 5 failed to neutralize the virus, the same positive and negative control

that the positive control serum in the previous experiment was tested against too large a dose of virus.

*Summary of all tests within the first year with the convalescent sera of individuals whose sera failed to protect in the first week of the disease*

*A. Paralytics.* Convalescent sera from 39 persons of various ages (Table VIII) were tested at frequent intervals after the onset of the disease. At 2 or 3 weeks after the onset, only 1 of 24

TABLE VII  
*Recheck of part of Experiment 5*

Patient's initials	Age	Type of case	Time after onset	Dilution of 5 per cent virus	Amount of virus	Amount of serum	Number of monkey	Results
	years				cc.	cc.		
Negative control.....	Normal monkey serum			1 : 20	0.13	0.60	J 260	Paralyzed 7 days
Negative control.....	Saline			1 : 20	0.13	0.60	J 274	Paralyzed 8 days
Positive control.....	Normal adult human serum (M.B.)			1 : 2	0.13	0.60	J 215	No paralysis
A. P.....	16	Non-paralytic	2 days	*	0.45	0.45	J 211	No paralysis
V. M.†.....	4	Paralytic	3 days	1 : 20	0.13	0.60	J 273	Paralyzed 9 days
V. M.....	4	Paralytic	7 months	1 : 20	0.13	0.60	J 277	Paralyzed 8 days
P. M.....	3	Non-paralytic	3 months	1 : 20	0.13	0.60	J 272	Paralyzed 9 days

\* Undiluted

† Bulbar case.

sera were retested. Whereas, in the previous experiment this positive control serum was tested against 0.2 cc. of a 5 per cent virus suspension, now 0.13 cc. of a 1:2 dilution of a 5 per cent suspension of the same batch of virus was used. As another positive control, the serum of a non-paralytic case (A. P.) which had previously protected was tested against a larger dose, 0.45 cc. of a 5 per cent virus suspension. In addition, 3 sera (V. M., 2 specimens, and P. M.) which had failed to protect were again tested with smaller amounts of virus than were used in the previous experiment. The results are shown in Table VII.

The positive control serum protected against the usual test dose of virus, while the negative control and the 3 other negative sera which were retested with smaller amounts of virus failed to do so. The serum from the non-paralytic case (A. P.) again protected. It appears, therefore,

sera protected; 7 of them were tested both at 2 and 3 weeks. Sera of 18 of the above individuals as well as of 12 others that failed to protect upon admission were retested once or twice at intervals of 1 to 6 months after the onset. Protection occurred in only 2 of these specimens, one, K. F., at 7 weeks, and the other, V. C., at 2½ and 3½ months. The latter showed protective substances for the first time on the 16th day.

At 7 to 9 months after the onset 20 of 21 sera tested failed to protect. The one serum that protected was obtained 9 months after the onset of the illness from K. F. who already had shown protective substance at 7 weeks. The other patient whose serum had developed protective substance previously was not available for retesting. The sera from 18 of the 21 individuals had been tested earlier during convalescence; 4 once, 12 two or three times and 2, four times always with

TABLE VIII

*Summary of neutralization tests during first year of convalescence of individuals whose sera failed to neutralize in the first week*

Age groups	Paralytic		Non-paralytic	
	Number tested	Number neutralized	Number tested	Number neutralized
<i>years</i>				
1-5.....	16	1	2	0
6-10.....	14	0	6	0
11-17.....	6	1	3	0
Adult.....	3	0	0	0
Total.....	39	2	11	0

negative results. The other 3 had been tested previously only in the acute stage of the disease.

*B. Non-paralytics.* Convalescent sera from 11 persons of various ages were tested at frequent intervals after the onset of the disease (Table VIII). Seven sera were tested 2 to 3 weeks after the onset and none neutralized the virus. Six sera, tested 1 to 3 months after the onset, showed no protective power; three of them had been tested previously during convalescence. Six sera tested 6 to 7 months after the onset also gave negative results; all but one of these had been tested earlier in convalescence.

*C. Cases with encephalitic symptoms.* The sera from two of these patients were tested and failed to show protective substances; one of them was tested 3 and 7 weeks, and 3 and 5 months after the onset, the other 6½ months after the onset.

*Summary of tests with convalescent sera from individuals whose sera protected in the first week of the disease*

The sera of 5 paralytics and 2 non-paralytics which showed protective power in the acute stage, when retested at later intervals still protected. The sera from two of the paralytics were retested in the second and third week respectively; two others at the end of 2 months; and the fifth after 6 months. One of the non-paralytics whose sera had protected on the 5th day, gave similar results in the 2d and 4th weeks, the other which had protected on the 4th day, also protected 6 months later. Thus, the ability of a serum to protect in the acute stage of the disease did not appear to be temporary, but was main-

tained for some time at least. However, no evidence of an increase in protective power was demonstrated either in two paralytics or in one non-paralytic. Serum from one of the paralytics had neutralized 0.1 cc. of a 5 per cent virus suspension in the acute stage, but failed to neutralize 0.2 cc. after 3 months. The other did not neutralize 0.1 cc. of a 5 per cent virus after 7 weeks, although a specimen obtained during the first week neutralized half that amount. A non-paralytic case also failed to show an increase of protective antibodies after 6½ months.

*Tests with a strain of virus isolated in 1935*

In order to compare the results with those obtained previously with the passage virus, two experiments were performed upon sera obtained in the acute and convalescent stages using the strain isolated from nasal washings.

*Experiment 7.* Ten specimens from 7 patients were tested; 3 were taken both in the acute and convalescent stages and 4 only in convalescence. None of these specimens had neutralized the F1 virus. The results, shown in Table IX, indicated that 2 of the 3 sera obtained soon after the onset and all of the 7 convalescent specimens failed to protect.

*Experiment 8.* Sixteen sera were tested. Six of these had previously protected against the passage virus. These included 3 specimens obtained from non-paralytics in the acute stage and 3 from paralytics during convalescence. Two of the latter (V. C. and K. F.) were from patients who showed protective substances both in the acute stage and in convalescence. Nine specimens obtained 5 to 9 months after the onset had failed to neutralize the F1 strain. Two of these were from non-paralytics and 7 from paralytics. The 16th serum was an acute-stage specimen of S. S. which appeared to protect against the recently isolated strain in Experiment 7 but had failed to neutralize the passage virus previously. The results are given in Table X. All 6 specimens which had protected against the passage virus also neutralized the recently isolated strain. The 9 convalescent sera which had failed to protect against the passage virus also failed to protect when the recently isolated strain was used. The specimen obtained from S. S. in the acute stage,

TABLE IX  
Neutralization tests with a strain of virus isolated during the 1935 outbreak

Patient's initials	Age	Type of case	Time after onset	Dilution of 5 per cent virus	Amount of virus	Amount of serum	Number of monkey	Results
	years				cc.	cc.		
Negative control.....	Normal monkey serum			1 : 2	0.13	0.60	J 252	No paralysis
Negative control.....	Normal monkey serum			1 : 10	0.13	0.60	J 253	Paralyzed 14 days
Negative control.....	Normal monkey serum			1 : 40	0.13	0.60	J 248	Paralyzed 8 days
Positive control.....	Normal adult human serum (A.G.)			1 : 2	0.13	0.60	J 72	No paralysis
P. B.†.....	5	Paralytic	3 days	1 : 10	0.13	0.60	J 249	Paralyzed 6 days
P. B.....	5	Paralytic	4 months	1 : 2	0.13	0.60	J 73	Paralyzed 15 days
P. B.....	5	Paralytic	4 months	1 : 10	0.13	0.60	J 241	Paralyzed 8 days
S. S.....	9	Paralytic	5 days	1 : 10	0.13	0.60	J 244	No paralysis
S. S.....	9	Paralytic	7 months	1 : 10	0.13	0.60	J 243	Paralyzed 8 days
R. M.....	4	Paralytic	5 days	1 : 2	0.13	0.60	J 258	Paralyzed 5 days
R. M.....	4	Paralytic	3½ months	1 : 2	0.13	0.60	J 257	Paralyzed 6 days
G. C.....	5	Paralytic	3 months	1 : 2	0.13	0.60	555	Paralyzed 13 days
G. C.....	5	Paralytic	3 months	1 : 10	0.13	0.60	J 242	Paralyzed 8 days
G. M.....	13	Paralytic	4 months	1 : 2	0.13	0.60	J 259	Paralyzed 6 days
J. C.....	8	Paralytic	7 months	1 : 10	0.13	0.60	J 246	Paralyzed 8 days
J. S.....	3	Paralytic	8 months	1 : 10	0.13	0.60	J 247	Paralyzed 12 days

† Bulbar case.

TABLE X  
Neutralization tests with a strain of virus isolated during the 1935 outbreak

Patient's initials	Age	Type of case	Time after onset	Dilution of 5 per cent virus	Amount of virus	Amount of serum	Number of monkey	Results
	years				cc.	cc.		
Negative control.....	Normal monkey serum			1 : 10	0.13	0.60	J 250	Paralyzed 8 days
Negative control.....	Normal monkey serum			1 : 10	0.13	0.60	J 271	Paralyzed 12 days
Negative control.....	Normal monkey serum			1 : 80	0.13	0.60	J 289	No paralysis
Positive control.....	Normal adult human serum (A.G.)			1 : 2	0.13	0.60	J 267	No paralysis
R. G.....	3	Non-paralytic	4 days	1 : 2	0.13	0.60	J 215	No paralysis
L. W.....	8	Non-paralytic	2 days	1 : 2	0.13	0.60	J 256	No paralysis
R. J.....	16	Non-paralytic	4 days	1 : 2	0.13	0.60	J 239	No paralysis
V. C.....	2	Paralytic	3½ months	1 : 2	0.13	0.60	J 290	No paralysis
E. F.....	4	Paralytic	3 months	1 : 2	0.13	0.60	J 266	No paralysis
K. F.....	13	Paralytic	8½ months	1 : 2	0.13	0.60	J 263	No paralysis
D. D.....	8	Non-paralytic	6 months	1 : 2	0.13	0.60	J 165†	No paralysis
E. B.....	11	Non-paralytic	5½ months	1 : 2	0.13	0.60	J 291	Paralyzed 7 days
K. S.....	11	Non-paralytic	6 months	1 : 10	0.13	0.60	J 278	Paralyzed 6 days
A. D.....	2	Paralytic	8½ months	1 : 10	0.13	0.60	J 284	Paralyzed 10 days
S. T.....	8	Paralytic	7 months	1 : 2	0.13	0.60	J 276	Paralyzed 7 days
H. P.....	9	Paralytic	9 months	1 : 2	0.13	0.60	J 280	Paralyzed 9 days
D. F.....	17	Paralytic	7½ months	1 : 2	0.13	0.60	J 264	Paralyzed 11 days
M. S.....	26	Paralytic	7½ months	1 : 2	0.13	0.60	J 262	Paralyzed 11 days
A. G.....	29	Paralytic	7 months	1 : 2	0.13	0.60	J 220	Paralyzed 9 days
K. G.....	31	Paralytic	7½ months	1 : 2	0.13	0.60	J 275	Paralyzed 7 days
S. S.....	9	Paralytic	5 months	1 : 10	0.13	0.60	J 286	Paralyzed 11 days

† This animal was resistant to poliomyelitis virus. The result was not included in the tabulations.

now failed to neutralize, a finding in keeping with that obtained with the passage virus.

These two experiments indicated that sera obtained in the acute and convalescent stages reacted similarly to both strains of virus. Three of 6 specimens obtained in the acute stage protected against both strains, and 3 failed to neutralize either strain. Three of 19 specimens obtained several months after the onset protected against both strains, whereas the other 16 neutralized neither strain of virus. The majority of convalescent sera thus failed to protect against a strain of virus obtained from the nasal washings of a patient in the same outbreak.

*Neutralization tests in which passage virus and sera obtained from 12 to 16 months after the onset of the disease were used*

*Experiment 9.* Thirty sera were tested, 24 from paralytic patients, 5 from non-paralytics and 1 from a patient who had the encephalitic form. Specimens taken from these patients during the acute stage and at intervals during the first year of convalescence had failed to neutralize the virus. The sera from two of the paralytic cases and one non-paralytic case taken 12 to 16 weeks after the onset neutralized the virus in duplicate tests. Thus, 3 of 30 patients apparently developed protective substances at the beginning of the second year following the illness. Two other persons whose sera protected during the acute stages of the disease were bled a year later in order to determine whether the neutralizing substances were still present. Both sera again protected.

#### DISCUSSION

Protective substances were found in 14 of 82 paralytic individuals in the first week of the disease; several of these patients had extensive paralysis. Kling and Levaditi (6), Flexner and Amoss (7), and Harmon and Harkins (15) also recorded instances in which protective substances were detected in the first week of illness. The first two authors (6, 7) thought that the detected neutralizing bodies had developed rapidly after the onset, while Harmon and Harkins (15) believed that they were present prior to infection. We concur with the latter opinion, for protective

substances were found very early in the disease and did not develop readily in convalescence. The presence of protective substances were demonstrated in the sera of two patients in the pre-paralytic stage, in one patient on the day paralysis developed, and in several others as early as one and two days after the onset. In one of the cases tested by Harmon and Harkins (15) neutralizing substance was also found in the pre-paralytic stage. On the other hand, when 39 of our 68 patients without protective substances in the acute stage were retested during the first year of convalescence, protective substances were found in the sera of only 2. Moreover, the sera of 2 individuals which had neutralizing power in the first week of the illness did not show a demonstrable increase during convalescence. Other observers also found that neutralizing substances did not develop readily during convalescence following paralysis (15, 19).

Non-paralytic cases showed protective substances in the acute stage of the disease more frequently (18 out of 32) than did paralytic cases. The neutralizing substances probably were present before the onset and did not develop rapidly after the disease set in. Their presence was detected as early as the first and second day of the illness. On the other hand, 11 of the 14 cases whose sera had no protective substances in the acute stage of the disease when retested several months later were still negative. This finding is in agreement with that of Paul and Trask (19) who found no neutralizing substance in a non-paralytic case several weeks and again one year after the onset.

The failure to develop protective substances in convalescent sera from paralytic cases also coincided with the findings of Paul and Trask (19) who showed that 6 of 7 sera taken 1½ to 10 weeks after the onset failed to neutralize the virus; 2 of them when retested a year later still failed to neutralize. On the other hand, Jensen (24) reported the presence of antibodies in paralytic and non-paralytic cases within a month after the onset. In a recent paper, however, Eagles and coworkers (25) stated that a pooled serum obtained from 67 paralytic patients approximately 4 weeks after the onset failed to neutralize the virus of poliomyelitis.

The failure of the majority of patients to de-

velop protective substances within 6 to 9 months after the onset is further confirmed by the negative results obtained with a strain of virus isolated from the same outbreak. Such findings differ from those of Howitt (22) who reported that convalescent serum more often neutralized a recently isolated strain than a passage virus. Likewise, Paul and Trask (19) found that convalescent sera from 6 of 7 paralytic cases neutralized a recently isolated strain, whereas only one serum neutralized the passage virus. It is possible that the strain used in the present work was more closely related to the passage virus than that used by the above workers (19, 22), for both strains of virus reacted similarly to 25 sera, 6 neutralizing and 19 failing to neutralize each strain. Moreover, 4 of 6 monkeys with residual paralysis that had recovered and were resistant to reinfection with F1 virus, were resistant also to the other strain. One of the 2 which failed to resist, developed only a mild attack with a rise in temperature and transitory weakness of the arms. On the other hand, the above mentioned investigators may have obtained neutralization more often with their recently isolated strains because they were less virulent than the passage strains. Howitt (22) indicated that the strain she used did not infect with regularity, while Paul and Trask (19) mentioned that their virus usually caused a less severe form of the disease than did the passage virus.

The absence of protective bodies during the first few months of convalescence in 48 of 50 paralytic and non-paralytic individuals whose sera failed to neutralize upon admission was demonstrated not only when 0.13 cc. of a 2.5 per cent (1:2 dilution of a 5 per cent virus suspension) was mixed with 0.6 cc. of serum, but also in many instances when tested against much smaller amounts of virus. Even though a number of sera were tested against 0.13 cc. of 5 per cent virus diluted 10 to 64 times, and in one instance 80 times, they still failed to neutralize. Smaller amounts of virus were used in testing these sera than most other investigators have employed. However, the intracerebral inoculation of a virus-serum mixture may not be suitable for the demonstration of neutralizing antibodies. Even

though diluted virus suspensions were used, the test still may be too severe.

A higher incidence of protective substances, 63 per cent, has been reported (Table I) in convalescent sera from paralytics obtained years after the disease, than was found in the present work in which the sera were collected within a year of onset. Our figure was approximately 22 per cent and included neutralizing substances found at the onset of the disease (17 per cent), and those which developed in convalescence (5 per cent). There have been no significantly large number of sera taken by any one group of investigators within one year of the onset of the disease for comparison with the results of the present work. The variations in technique would hardly explain the different results, for other workers frequently obtained neutralization although larger amounts of virus suspension were used. Howitt (20b) reported that pooled convalescent sera from long standing cases had a higher titer of neutralizing substances than those from recently recovered cases. In this investigation, the sera of 24 paralytics which had failed to neutralize during the first 9 months of convalescence, were retested 12 to 16 months after the onset. Two of these specimens obtained 14 months after the onset from children 7 and 9 years of age neutralized for the first time. It is also possible that more of these individuals may subsequently develop neutralizing substances. If this does occur, is it due to the slow development of neutralizing substances, is it produced by further exposure to the virus resulting in hyperimmunization, or can it appear as a result of nonspecific factors as Jungeblut and Engle (26) have suggested? It is noteworthy that the sera which neutralized at the beginning of the second year after the onset were obtained in October 1936. However, there was practically no poliomyelitis in New York City during 1936. The children from whom the sera were obtained had not been in a locality where poliomyelitis was prevalent.

Until recently the presence of neutralizing substances in the serum was accepted as an index of immunity to poliomyelitis. Certain experimental and clinical observations, however, do not support this view. It has been shown (27, 28) that sera of monkeys injected subcutaneously with a



subinfective dose of virus may after a few weeks neutralize poliomyelitis virus *in vitro*, even though the animals are not able to resist an intracerebral or intranasal instillation of potent virus. Further, convalescent monkeys which are refractory to reinoculation may show no neutralizing substance in their sera (29, 30, 31). Evidence has been presented previously to support the contention that protective substances were present in 14 of 82 paralytic patients when the disease developed. There was no evidence to indicate that the paralysis was less extensive in the 14 persons whose serum neutralized in the acute stage than in the 68 whose serum failed to do so. Only 2 of the 14 had mild paralysis, that is a partial involvement of one limb, 2 had bulbar involvement, 2 others showed extensive paralysis of one leg. The remaining 8 had involvement of 2 extremities or more. Of the 68 whose serum failed to neutralize in the acute stage, 8 had a mild paralysis, 13 had involvement of one leg and 9 cases were bulbar. The remaining 38 had a muscular paralysis in at least two extremities. The majority of sera tested from the convalescent paralytic patients without protective substances in the first week of the disease did not develop any as late as 12 to 16 months after the onset of the disease. Nevertheless, second attacks of poliomyelitis are rare. Although one cannot always interpret human disease in the light of animal experimentation, one may assume an immunity in the majority of convalescent poliomyelitis patients in the absence of protective substances in the serum.

On the other hand, the incidence of protective substance in non-paralytic patients is greater than that in paralytics and approximates that in normal individuals of the same age. In the acute stage of the disease in non-paralytic patients its presence may help to limit the spread of the virus, while in the paralytics its frequent absence may be a factor accounting for the widespread involvement in some cases. Very noteworthy was the absence of neutralizing substances in the specimens of serum taken on admission and one year later from a paralytic boy who had had a previous attack of poliomyelitis in 1933 with residual paralysis.

Consequently, it appears that there must be factors other than the presence or absence of protective substances in the serum that determine

the resistance of the host to poliomyelitis. Indeed, in interpreting these results, we must consider the possibility that the neutralizing substances are not specific, that is, they may develop irrespective of exposure to the virus. The immunity present in the absence of neutralizing substances may be cellular rather than humoral.

The presence or absence of neutralizing substances in the acute and convalescent stages was not related to the degree of recovery from paralysis, a finding in agreement with that of Harmon and Harkins (15). Howitt (12), on the other hand, indicated that some correlation did exist between these two factors. We were unable to find that the 14 paralytic cases who had neutralizing substances in the acute stage improved more rapidly than the other 68 who had none. Indeed, in three of the 14, the paralysis was progressive. Of the two paralytic individuals (Table VIII) who developed demonstrable neutralizing substances within the first 9 months of convalescence, one showed considerable recovery of muscular power in several months, while the other improved slowly although a progressive increase in neutralizing substances was demonstrated after the 16th day. In 16 of 20 whose sera had failed to neutralize after 7 to 9 months, considerable clinical improvement occurred. One bulbar case and two spinal-paralytic cases made a complete recovery within a month after the onset.

The protection test offers no aid in the diagnosis of non-paralytic poliomyelitis. In the acute stage the proportion of sera from patients over the age of 5 that neutralized approximated that for normal individuals. Further, 11 of those whose sera showed no neutralizing power in the acute stage did not develop any within 6 to 7 months, thereby failing to give any serological evidence that the diagnosis was correct. Clinically, all the cases had the typical type of onset and symptoms, including pain and stiffness of the neck and back with pleocytosis of the cerebrospinal fluid. The lack of serological evidence does not invalidate the diagnosis, inasmuch as the majority of cases with frank paralysis also failed to develop protective substances.

#### SUMMARY

1. Neutralization tests carried out with the F1 strain (monkey passage) of virus on sera ob-



tained from paralytic and non-paralytic cases during an outbreak of epidemic proportions gave the following results: (a) Of 82 paralytic cases tested during the acute stage of the disease, the sera of 14 neutralized the virus; (b) 4 of these patients were tested in the preparalytic stage, and the sera of 2 possessed protective bodies; (c) of 32 non-paralytic cases tested in the acute stage of the disease, the sera of 18 neutralized; (d) of 3 encephalitic cases tested in the acute stage, the serum of one neutralized; (e) during convalescence only 2 of 39 paralytics who had no protective substances in the acute stage developed them within a few months after the onset. Two of 24 individuals whose sera had failed previously to neutralize in convalescence developed protective substances 12 to 16 months after the onset; (f) neutralizing substances which had not been present in the acute stage in 11 non-paralytics also failed to develop in the sera tested several months after the onset. One of 5 obtained 12 to 16 months after the onset neutralized; (g) the sera of 2 cases with encephalitic symptoms likewise failed to protect 5 to 6 months after the onset. One which was obtained 12 months after the onset was also negative; (h) two paralytics and one non-paralytic, whose sera neutralized in the acute stage of the disease, failed to show a demonstrable increase of neutralizing substances 2 months or longer after the onset.

2. Sera of 9 of 18 so-called normal individuals, over the age of 10, neutralized.

3. A strain of virus isolated from the outbreak from which the bloods were collected was tested against the sera and gave results comparable to those obtained with the passage (F1) virus.

4. No evidence of a definite relationship was found between the presence of protective substances in serum and (1) resistance to poliomyelitis, (2) the diagnosis of the non-paralytic form of poliomyelitis and (3) the degree of recovery from paralysis.

The authors wish to express their thanks to Dr. Thomas M. Rivers and to Dr. Morris Schaeffer for their valuable suggestions during the course of this work.

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# MACROCYTIC ANEMIA IN PREGNANT WOMEN WITH VITAMIN B DEFICIENCY<sup>1</sup>

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(Received for publication August 17, 1936)

Recent observations of sprue (1), pernicious anemia (2), tropical macrocytic anemia (3) and the pernicious anemia of pregnancy (4) have led to the hypothesis that a dietary deficiency of vitamin B<sup>3</sup> or some closely allied substance is responsible both for the anemia and for the clinical manifestations of these diseases. Animal experiments designed to test this hypothesis (5, 6, 7) have yielded results which vary with the species of animal employed. This and the fact that macrocytic anemia does not occur in animals as a spontaneous entity constitute limitations which made it seem desirable to study the problem in human beings. The occurrence in pregnancy of a syndrome which resembles pernicious anemia and the other macrocytic anemias in every important respect except that it is temporary suggested that there may develop during that period a temporary deficiency of factors necessary to prevent macrocytic anemia. If the assumption is correct that vitamin B is the responsible factor one would expect to encounter a high percentage of anemia of this type in women who, by force of circumstances, were taking a diet during pregnancy which was deficient in vitamin B. Pregnant patients taking poor diets have been observed by Strauss and Castle (8) to develop macrocytic anemia late in pregnancy. The subjects of that investigation consumed diets deficient in many substances, however, and it was impossible to be certain whether one alone of the missing factors was responsible for the development of the anemia. It seemed desirable, therefore, to determine whether pregnant women such as those

studied by Strauss and Castle would develop this form of anemia when their habitually inadequate diet was supplemented to bring it to the level of full caloric requirements and otherwise adequate conditions but without any attempt to supply deficiency of vitamin B. It may be noted that these patients admitted to an obstetrical clinic were subsisting on a diet deficient in calories as well as vitamins. Any resulting anemia under these circumstances may be attributable to general nutritional deficiency or to vitamin inadequacy. It was the purpose of this study to determine which factor is significant. Every other known essential was therefore added to the diet and such amounts of vitamin B as had been taken in the previous diet continued to be given but no attempt was made to increase this factor, the importance or unimportance of taking which was the object of the study. By careful clinical observation the early signs of vitamin B deficiency previously described in a report from this Clinic (9) were sought and when detected were promptly relieved by the administration of brewer's yeast or liver extract. The present communication is a report of studies made chiefly upon the blood, gastric secretion and clinical manifestations of these women from the 4th month of pregnancy to term.

## METHODS OF STUDY

The subjects of the study were selected from among the patients attending the Outpatient Maternity Clinics of this and the Pennsylvania Lying-In Hospitals. Each subject was examined every 10 to 14 days at which time a detailed record was made of symptoms and physical observations. Blood was drawn without stasis from an antecubital vein.

Seven clinical phenomena, manifestations of vitamin B deficiency, recognizable from previous study (9) were watched for in particular. The

<sup>1</sup> Aided by a donation from Mr. Samuel S. Fels and by a grant from the Faculty Research Committee of the University of Pennsylvania.

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<sup>3</sup> The term vitamin B as used in this paper includes all members of the vitamin B complex. Individual members are referred to as vitamin B<sub>1</sub>, B<sub>2</sub>, etc.

changes were: alterations of the tongue, gastrointestinal symptoms, paresthesias, impaired vibratory sense, susceptibility to fatigue, edema and tachycardia. The term "deficiency index" has been used in the text to denote the number of these phenomena manifested at any given time by each individual. The deficiency index ranges from 0 to 7.

The acuity of perception of vibration was determined with a C tuning fork (128 vibrations per second). A standard of reference was obtained from normal persons who perceived a sense of vibration for 20 to 30 seconds when the fork, vibrating at maximal intensity, was placed over

were made in duplicate from smears of blood stained immediately with brilliant cresyl blue, 1000 cells being counted. Morphological changes in the cells were observed from smears of blood prepared with Wright's stain. The infants were examined and erythrocyte counts and hemoglobin estimations were made at birth and at 1, 3 and 9 months of age.

## RESULTS

### *Dietary requirements*

The 11 subjects of this report took diets so constant that the composition, the caloric and the protein values could be estimated with accuracy.

TABLE I  
*Chart showing articles composing the diets of Group I*

<i>Vegetables</i>	<i>Cereals and starches</i>	<i>Pastry and desserts</i>	<i>Meat</i>
Beans (kidney)	Cornmeal	Bread	Veal
Potatoes	Cream of wheat	Crackers	Lamb
Turnips	Cornflakes	Cake	Corned beef
Corn (canned)	Rice	Pie: apple	Frankfurters
Beets	Macaroni	pear	
Spinach	Spaghetti	Puddings: cornstarch } without rice } milk gelatine }	
Onions		Candies	
Cabbage		Jellies	
Rutabaga			
Carrots			
<i>Fats</i>	<i>Seasonings, etc.</i>	<i>Fruit</i>	<i>Beverages</i>
Butter	Chocolate	Apples	Tea
Mayonnaise	Cinnamon	Pears	Coffee
Lard	Molasses	Grapes	Grape juice
	Syrup	Cocoanut	Gingerale
	Honey, salt, etc.		

any bony prominence of the lower extremities. Electrocardiograms and orthodiagrams were made several times, as were also gastric analyses in which the rate and the acid content of the fasting secretion and the acid response to 50 cc. of 7 per cent alcohol were determined. Titrations of acid were carried out in the usual way with 0.1 normal NaOH, using Topfer's reagent and phenolphthalein as indicators.

Erythrocyte counts were made in triplicate, using pipettes certified by the U. S. Bureau of Standards. Hemoglobin values were determined by the method of Stadie (10) which permits accurate determination of hemoglobin concentration to within 0.3 gram per 100 cc. of blood. Hematocrit determinations were made in duplicate using Wintrobe tubes. Mean corpuscular volume and mean corpuscular hemoglobin were calculated in the usual fashion (11). Reticulocyte counts

Eight subjects hereafter called Group I consumed throughout the period of observation a diet composed of the articles shown in Table I. These foods were taken in amounts which supplied approximately 50 calories and 1.5 grams of protein per kilogram of body weight per day. The vitamin B:calorie ratio of this diet was approximately 1.66. The vitamin B per calorie requirement of these subjects at the beginning of the observation, calculated from Cowgill's <sup>4</sup> formula (12), averaged 1.5 (Table II). Since the caloric intake of these women remained essentially constant throughout observation, their theoretical re-

$$^4 \frac{\text{Vitamin B}}{\text{Calories}} = .0000284 \text{ Weight grams.}$$

Although the figure obtained by this formula of Cowgill's applies strictly to vitamin B<sub>1</sub>, it is used here as an index of the concentration of other vitamin B fractions since, as far as is known, other fractions were present in the diet in approximately the same concentration as B<sub>1</sub>.

TABLE II

*The theoretical requirement of vitamin B per calorie,\* the intake of vitamin B per calorie\*\* and the deficiency index of subjects of Group I at various times during pregnancy*

Sub- ject num- ber	Beginning of period of observation			Appearance of definite clinical pheomemo			Finol exomination after administration of therapy††			
	Days of preg- nancy	Vitamin B: calorie ratio required	D. I.†	Days of preg- nancy	Vitamin B: calorie ratio required	D. I.†	Days of preg- nancy	Vitamin B: calorie ratio required	D. I.†	Days of ther- apy
1	82	1.8	0	236	2.0	7	271	2.1	2	35
2	102	1.5	0	264	1.6	4	280	1.6	2	16
3	112	1.5	0	234	1.7	6	269	1.7	1	35
4	06	1.3	0	233	1.6	5	277	1.6	1	44
5	107	1.6	0	249	1.9	5	277	2.0	3	28
6	96	1.5	0	249	1.8	3	277	1.8	0	28
7	62	1.4	0	232	1.7	7	280	1.8	1	48
8	138	1.8	0	265	2.2	6	279	2.2	1	14
Average	99	1.5	0	245	1.8	5	276	1.9	1	31

\* From the Cowgill formula (12):  $\frac{\text{Vitamin B}}{\text{Calories}} = .0000284$

Weight grams.

\*\* Vitamin B per calorie of diet = 1.66.

† D. I. = deficiency index.

†† Vitamin B per calorie of diet + therapy = 6.55.

quirement for vitamin B increased in proportion to the increase in weight. On the average, at the 245th day of pregnancy this theoretical requirement exceeded the vitamin B per calorie content of the diet. At approximately this same time, also, definite clinical evidence of vitamin B deficiency was observed and the characteristic blood changes to be described were noticed. As soon as these changes became clearly recognizable 21 grams of brewer's yeast<sup>5</sup> were administered orally or 2 cc. of liver extract<sup>6</sup> were given intramuscularly daily until the termination of pregnancy. These materials increased the vitamin B per calorie intake to 6.55, far exceeding the vitamin B requirement. Following this procedure all clinical evidence of deficiency disappeared and blood values returned toward normal.

Group II includes 3 subjects who habitually consumed a normal varied diet, having an average vitamin B: calorie ratio of 2.8 (Table III). The vitamin B per calorie requirement of this group at no time exceeded the vitamin B content of the diet and no definite clinical evidence of deficiency or characteristic alterations in the blood were encountered.

<sup>5</sup> This material was kindly supplied as "Brewer's Yeast-Harris" through the courtesy of the Harris Laboratories, Tuckahoe, N. Y.

<sup>6</sup> Liver extract no. 343 (Lilly) for intramuscular use.

TABLE III

*The theoretical requirement of vitamin B per calorie, the intake of vitamin B per calorie and the deficiency index of subjects of Group II at various times during pregnancy*

Subject number	Vitamin B: calorie ratio of diet	Beginning of period of observation			8th to 9th month			Final examination		
		Days of pregnancy	Vitamin B: calorie ratio required	D. I.	Days of pregnancy	Vitamin B: calorie ratio required	D. I.	Days of pregnancy	Vitamin B: calorie ratio required	D. I.
1	3.4	59	1.3	0	220	1.5	0	273	1.5	0
2	2.5	125	1.5	0	224	1.7	0	273	1.8	0
3	2.3*	120	1.5	0	246	1.9	3	267	1.9	0
Average	2.8	101	1.4	0	230	1.7	1	271	1.7	0

\* 21 grams of brewer's yeast administered to this subject from the 253d to the 267th day of pregnancy increased to 10.2 the Vitamin B: calorie ratio of her diet during that time.

Adequate intake of vitamins A, C and D and of iron, in addition to adequate protein and total calorie intake, was assured for both groups by the daily addition to the diet of 150 cc. of orange juice, one tablet of halibut liver oil concentrate with viosterol<sup>7</sup> and 6 grains of ferrous sulphate.<sup>8</sup>

### Changes in the blood

The following characteristic changes in the blood developed during pregnancy in all of the subjects of Group I: decrease in the number of red blood cells, increase in mean cell volume and in mean cell hemoglobin, macrocytosis, reticulocytosis, and the appearance of many polychromatic cells, poikilocytes and young white cells. These changes were greatest at the time that the diet became theoretically inadequate in vitamin B and when the clinical manifestations were definite. The maximal changes in blood values of the subjects of this group are given in Table IV. The average number of red blood cells decreased 18 per cent and the mean cell volume increased 21 per cent. There was a slight fall in hemoglobin early in the period of observation which did not equal the fall in red cells, so that the mean cell hemoglobin increased on the average 14 per cent. The mean cell hemoglobin increased further in certain subjects later in the

<sup>7</sup> Obtained from E. R. Squibb and Sons.

<sup>8</sup> Feosol was kindly supplied by the Smith, Kline and French Co., Phila.



period of observation but before vitamin B therapy was begun. This was due to a return toward normal of the total hemoglobin values without in-

crease in the number of red cells. Alterations in the blood first appeared 1 to 3 months after observations were begun, concomitant with the first appearance of clinical evidence of deficiency (Figures 1, 2 and 3). These changes were at first slight, becoming clearly recognizable only later at which time therapy was begun. The percentage of reticulocytes gradually increased until the administration of vitamin B was commenced, the average maximum being 3.5 per cent. Distinct variation in size of the red cells was noticeable at this time (Figures 4 and 5). Pale staining macrocytes were frequent, as were poikilocytes, polychromatic cells and immature white cells.

#### Clinical observations

The symptoms and physical signs which appeared in the subjects of Group I during the period of observation were characteristic (Table V). Alterations in the tongue and neurological changes occurred in all subjects, the other characteristic signs being less constant. The intensity

TABLE IV

Maximal change in blood values of subjects of Group I

Subject number	Initial blood values					Blood values at time of greatest anemina				
	R. B. C.	Hgb.	Hemato- crit	M. C. V.*	M. C.† Hgb.	R. B. C.	Hgb.	Hemato- crit	M. C. V.	M. C.† Hgb.
	mil- lions	grams per 100 cc.	vol- umes per cent	$\mu^3$	grams $\times 10^{-12}$	mil- lions	grams per 100 cc.	vol- umes per cent	$\mu^3$	grams $\times 10^{-12}$
1	3.65	13.0	37.0	104	35.6	3.01	11.3	37.4	124	37.5
2	4.28	13.1	40.4	91	30.7	3.29	11.9	38.9	118	36.3
3	4.74	13.2	41.2	93	27.8	3.87	14.0	45.3	117	36.2
4	3.33	10.9	35.0	107	32.0	2.99	11.6	38.3	128	38.7
5	3.90	12.6	38.2	98	32.2	3.14	12.3	38.1	121	39.2
6	3.79	12.8	39.2	103	33.8	2.95	11.0	35.6	121	37.2
7	4.00	13.5	39.7	90	33.8	2.81	11.3	34.7	124	40.3
8	3.76	13.4	40.1	107	35.5	3.70	12.9	42.2	111	33.9
Average	3.93	12.8	39.0	100	32.7	3.23	12.0	38.8	121	37.4
Per cent of change						-18.0	-6.0	-0.5	+21.0	+14.0

\* M. C. V. = Mean corpuscular volume.

† M. C. Hgb. = Mean corpuscular hemoglobin.

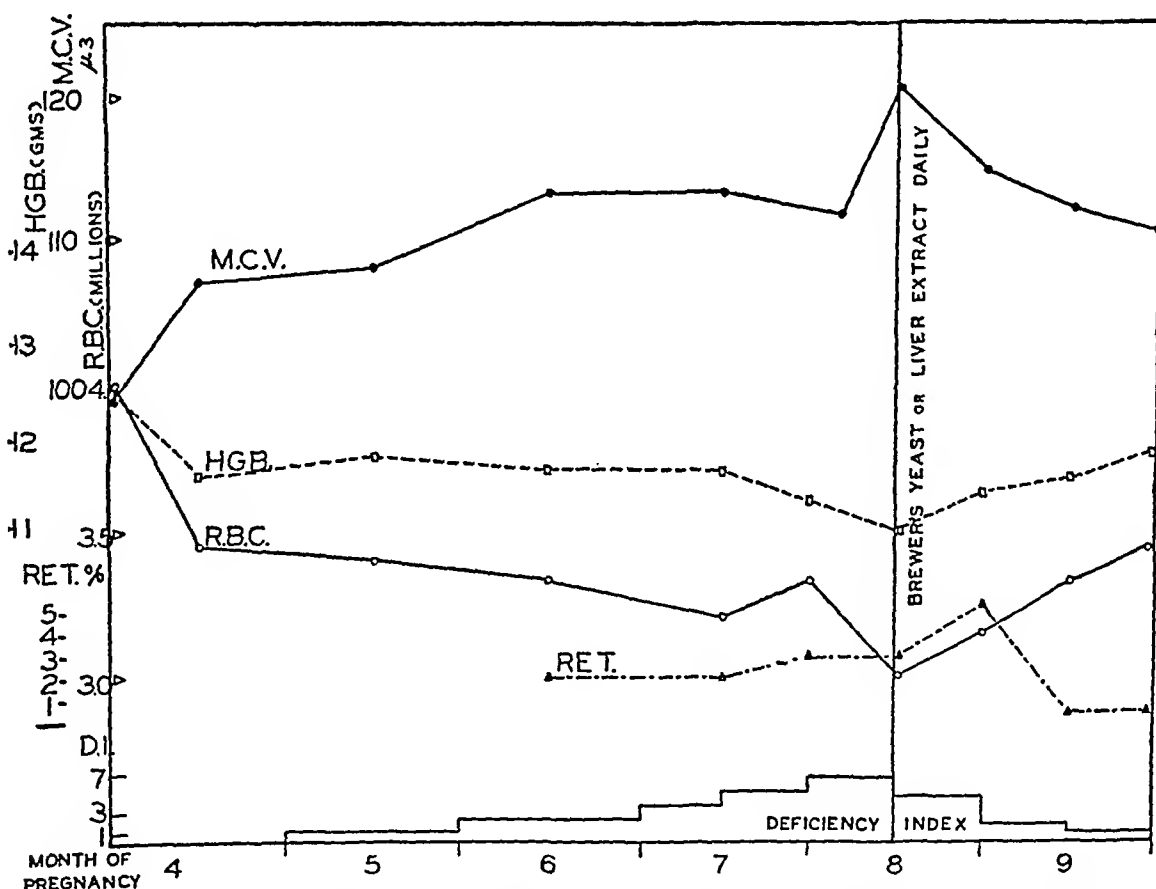


FIG. 1. CHANGES IN AVERAGE BLOOD VALUES OF 3 SUBJECTS OF GROUP I WHO RECEIVED THERAPY DURING THE 8TH AND 9TH MONTHS OF PREGNANCY.

Ret. = Reticulocytes. M. C. V. = Mean corpuscular volume. D. I. = Deficiency Index.

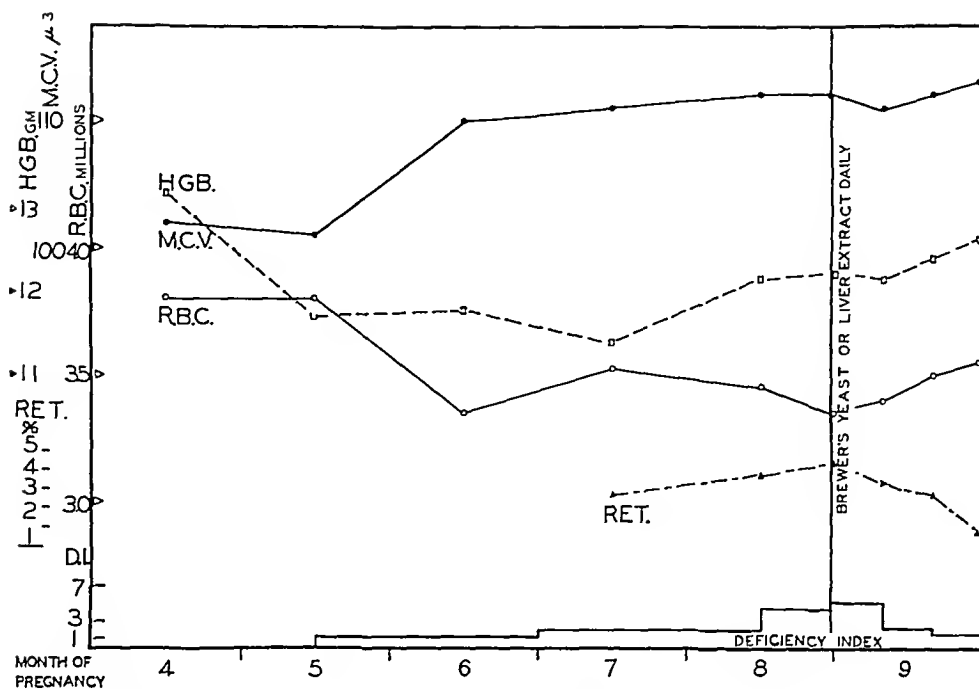


FIG. 2. CHANGES IN AVERAGE BLOOD VALUES OF 2 SUBJECTS OF GROUP I WHO RECEIVED THERAPY DURING THE 9TH MONTH OF PREGNANCY.

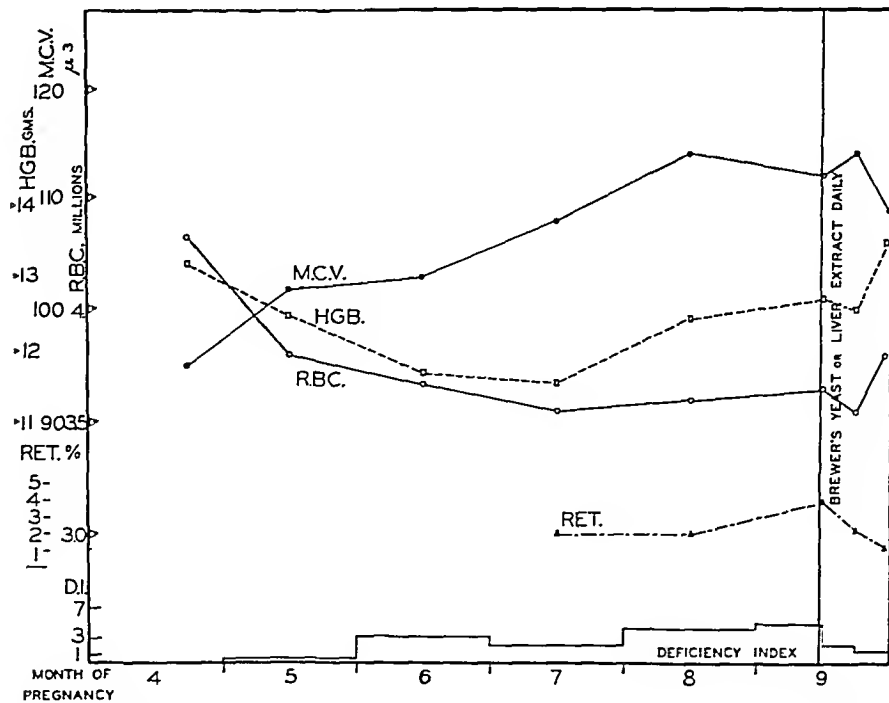


FIG. 3. CHANGES IN AVERAGE BLOOD VALUES OF 2 SUBJECTS OF GROUP I WHO RECEIVED THERAPY DURING THE LAST 2 WEEKS OF PREGNANCY.

TABLE V

*Clinical observations on subjects of Group I: Their incidence, and time of relief following vitamin B therapy*

Clinical observations	Number of subjects affected	Average time of relief after therapy
		<i>days of therapy</i>
Tongue changes.....	8	14 (5-39*)
Paresthesias.....	7	18 (9-28)
Susceptibility to fatigue.....	6	14 (9-25)
Edema.....	5	26 (9-35)
Gastro-intestinal symptoms.....	5	17 (7-25)
Blood changes.....	8	7† (5-14)
Impaired vibratory sense.....	8	24 (17-35)
Tachycardia.....	4	19 (9-25)

\* Figures in parentheses represent maximum variations from the average.

† This figure represents time of first definite change in reticulocyte count.

of the manifestations varied among the individuals (for details see protocols), but once present each symptom or physical sign, at first so mild as to escape casual observation, persisted, usually increasing until therapy was begun (Figures 1, 2 and 3). The first changes noticed were those in the tongue. These changes started at the edges

of the tongue and later spread over the dorsum; they varied from slight reddening, with or without loss of papillae, to ulceration. The ulcers were shallow, at first discrete, later coalescing, with smooth red bases and white aphthoid edges. They were painful, and in one of the subjects were associated with ulcers of the lips. Changes in the tongue always preceded gastro-intestinal symptoms and occurred at approximately the time when the women first complained of being easily fatigued. The earliest gastro-intestinal symptom was anorexia, followed by heartburn or a sense of constant fullness in the epigastrium. Dysphagia was present in 2 subjects. Nausea and vomiting were rare. Constipation was the rule, but abdominal cramps with intermittent diarrhea occurred twice.

Edema, extensive in one instance, was manifest in most subjects as slight pitting on pressure over the tibiae. An average increase in the pulse rate of 16 per minute occurred. The cardiac rate frequently was unstable. Prominence of the pulmonary artery was seen on orthodiagraphy in one-third of the subjects. No characteristic change in resting blood pressure was observed.

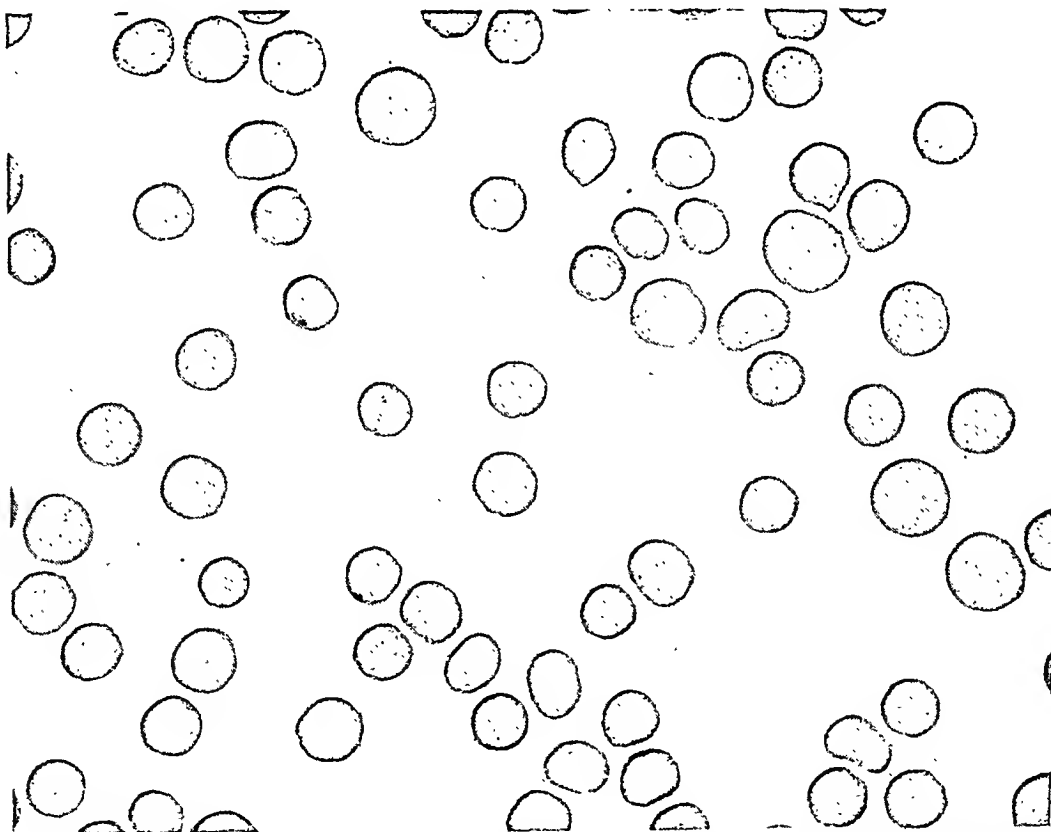


FIG. 4. PHOTOMICROGRAPH OF THE BLOOD OF SUBJECT NUMBER 7 AT THE TIME OF MAXIMAL ANEMIA.  $\times 774$ . WRIGHT'S STAIN.

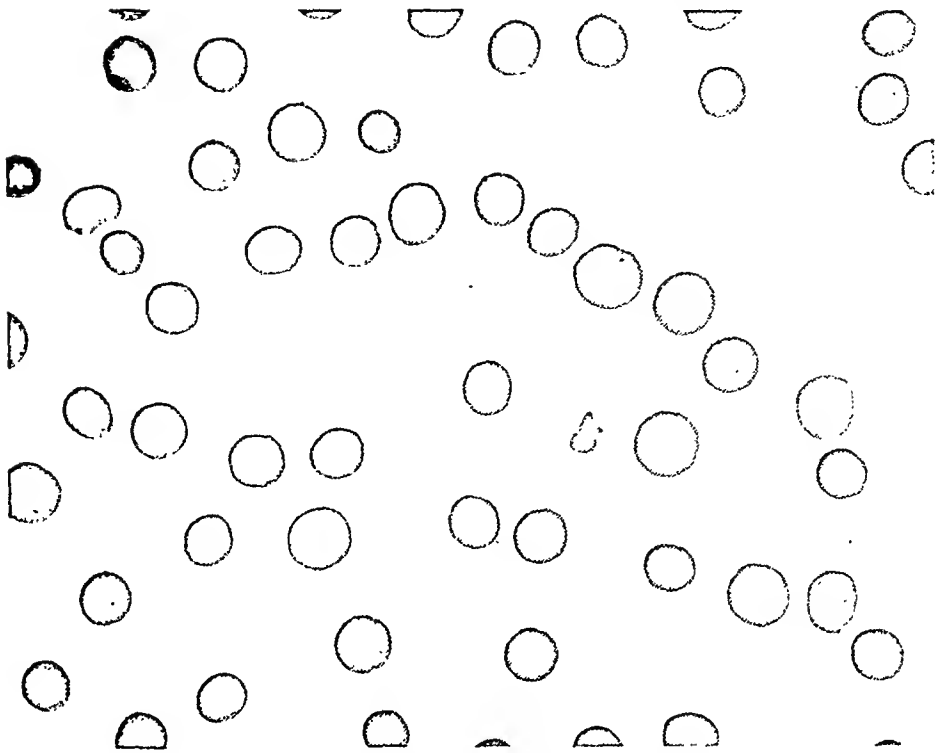


FIG. 5. PHOTOMICROGRAPH OF THE BLOOD OF SUBJECT NUMBER 1 AT THE TIME OF MAXIMAL ANEMIA.  $\times 774$ . WRIGHT'S STAIN.

Impairment of vibratory sensation, which was always preceded by paresthesias, occurred to some degree in the lower extremities of all patients. Decrease in the duration of the perception was the first change noticed. This gradually progressed until, in some instances, the sense of vibration was temporarily lost: first in the great toe, and, in certain subjects, in all the toes and over the malleoli and tibiae.

#### *Gastric acidity and rate of secretion*

No characteristic change in gastric acidity developed in either group. In 3 individuals of Group I it decreased, in 3 it increased and in 2 no change occurred (for details see protocols). The rate of secretion was quite constant, the average figure for all subjects being 44 cc. per hour both before and after therapy.

#### *Response to therapy*

The administration of brewer's yeast or liver extract produced relief from all characteristic changes observed (Figures 1, 2, 3). No sig-

nificant difference in the effectiveness of these two therapeutic agents was noticed. The percentage of reticulocytes decreased promptly to a level below that observed at any time during the preceding period of observation, and other young forms disappeared from the blood. In some subjects the decline in reticulocytes was preceded by a brief rise, the maximal count observed being 5.6 per cent. The mean corpuscular volume and the mean corpuscular hemoglobin declined steadily; the red cells and the total hemoglobin increased. The blood values returned most nearly to normal in those subjects who received the longest course of therapy. An independent increase in total hemoglobin occasionally took place before yeast or liver therapy was begun; this was attributed to the administration of iron with the diet.

The clinical disturbances also responded promptly to therapy (Table V). Improvement in lingual changes and freedom from fatigue were the first effects noticed, on the average, 14 days after therapy was begun. In general, the phenomena first to appear were first to disappear.

Before delivery, all the subjects were free of significant symptoms and physical signs, except for slight impairment of vibratory sense in two subjects and for edema in one.

All subjects were delivered of full term normal infants who have developed normally.

### *Observations on Group II*

No striking changes were observed in the blood values of Group II (Table VI, Figure 6). In the late months of pregnancy a 7 per cent increase in mean corpuscular volume occurred and occasional macrocytes appeared in the blood smear (Figure 7). Subject 3, whose theoretical requirement of vitamin B had increased more than

TABLE VI

### *Maximal change in blood values of Group II*

Subject number	Initial blood values					Blood values at time of maximal change				
	R. B. C.	Hgb.	Hemato- crit	M. C. V.	M. C. Hgb.	R. B. C.	Hgb.	Hemato- crit	M. C. V.	M. C. Hgb.
	mil- lions	grams per 100 cc.	vol- umes per cent	$\mu^3$	grams $\times$ $10^{-12}$	mil- lions	grams per 100 cc.	vol- umes per cent	$\mu^3$	grams $\times$ $10^{-12}$
1	3.97	13.3	38.7	98	33.5	3.81	13.1	41.1	109	35.1
2	4.47	14.2	42.0	91	34.7	4.56	13.4	45.3	99	29.3
3	3.82	13.2	39.9	101	34.5	3.86	13.7	42.5	110	35.5
Average	4.09	13.6	40.2	99	33.2	4.08	13.5	43.1	106	33.3
Per cent of change						-0.2	-0.7	+7.0	+7.0	+0.3

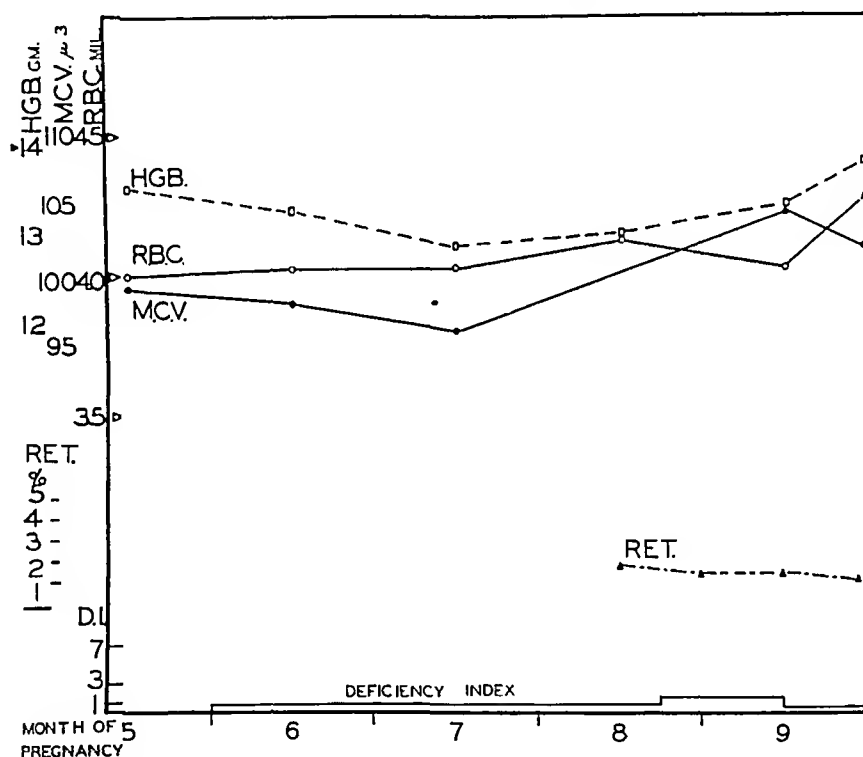


FIG. 6. AVERAGE BLOOD VALUES OF SUBJECTS OF GROUP II.

that of other members of the group, developed sore tongue and paresthesias, clinical disturbances similar to the earliest shown by the subjects of Group I. Both the clinical disturbances and the blood changes in this woman responded promptly to the administration of brewer's yeast. It must be concluded that the diet of this individual, though theoretically adequate, failed in the last months of pregnancy to meet entirely the increased requirement of vitamin B. This suggests that the optimum requirement is, in some in-

stances, greater than that indicated by the theoretical calculations.

### DISCUSSION

The changes in the blood observed in Group I were characteristic of those known to exist when the bone marrow is hyperplastic, viz., moderate anemia, associated with the presence of immature cells. A substance necessary for normal bone marrow activity, lacking in the habitual diet, was supplied by yeast or by liver extract. That the

return toward normal blood values was dependent upon the administration of that substance and was not a spontaneous improvement, such as sometimes occurs late in the microcytic anemia of pregnancy (13), is shown by the fact that improvement took place only after therapy was begun, regardless of the time in pregnancy, and that

acteristically seen in pernicious anemia, sprue, tropical macrocytic anemia, pernicious anemia of pregnancy and the mild macrocytic anemia of pregnancy observed by Strauss and Castle. In the present observations these changes took place during a period when the intake of food containing vitamin B was inadequate and were relieved

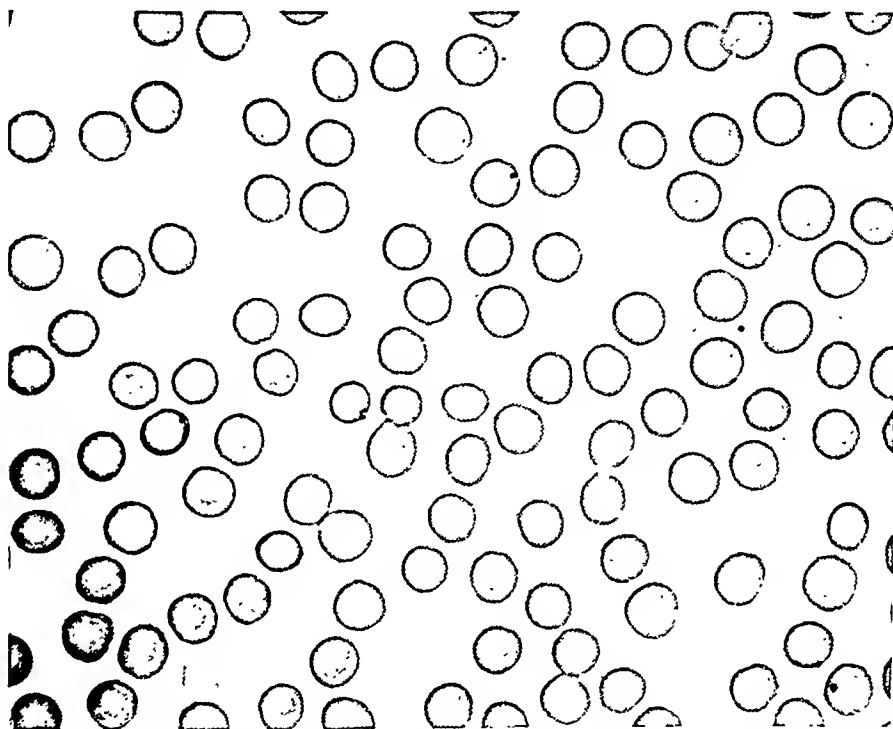


FIG. 7. PHOTOMICROGRAPH OF THE BLOOD OF SUBJECT NUMBER 2 OF GROUP II.  $\times 774$ . WRIGHT'S STAIN.

the return toward normal was greatest in those subjects who received the longest course of therapy.

The clinical disturbances in the subjects of Group I formed a characteristic syndrome involving chiefly the gastro-intestinal tract and the nervous system and were similar to those observed in a previous study of clinical vitamin B deficiency (9). The individual variation in intensity of these manifestations was interesting and suggests either that minor changes in the diet or environment or individual differences in susceptibility may alter considerably the manifestations of a given deficiency.

Both the clinical disturbances and the alterations in blood values were analogous to those char-

by therapeutic agents known to be rich in vitamin B. Further identification of the substance responsible for the changes noticed must remain incomplete until various factors now grouped together as vitamin B are identified and separately tested.

It is interesting to observe that the entire syndrome occurred without consistent change in gastric acidity. This suggests that, while altered gastric secretion of acid may be a late result of deficiency, it is not the cause of the syndrome.

The close correlation of a calculated deficiency in vitamin B with the actual deficiency suggests that the formula of Cowgill indicates approximately the sufficiency of the diet in vitamin B. The vitamin B requirement appears to increase

during pregnancy with gain in weight in accordance with this formula, a fact, which as demonstrated by the present study, has suggested the important inference that a diet, adequate in vitamin B at the outset of pregnancy, may fail to meet the increased demand for that vitamin late in pregnancy.

#### SUMMARY

1. Characteristic hematological changes and clinical phenomena which developed in 8 pregnant women have been related to a diet adequate except for the amount of vitamin B which it contained.

2. The blood changes were: decrease in number of erythrocytes, increase in mean corpuscular volume and mean corpuscular hemoglobin, macrocytosis, reticulocytosis, the appearance in the blood smear of many polychromatic cells, of poikilocytes and of immature white cells.

3. The clinical disturbances encountered were: glossitis and ulceration of the tongue, impairment or loss of vibratory sense, tachycardia, edema, gastro-intestinal symptoms, easily induced fatigue and paresthesias.

4. The blood and clinical changes responded to brewer's yeast orally or to liver extract intramuscularly.

5. The clinical disturbances appeared, in general, when calculations based on Cowgill's formula indicated a deficiency in vitamin B.

I am indebted to the members of the Obstetrical Staffs and of the Social Service Departments of the Pennsylvania Lying-In Hospital and of this hospital for their cooperation. I am indebted also to members of the Edward B. Robinette Foundation for their assistance in taking electrocardiograms and orthodiagrams and to Dr. J. Harold Austin for his aid in preparation of the manuscript. The assistance of Miss I. M. Dworak, of the Dietetic Division of this hospital, in calculating the dietary requirements of the subjects is gratefully acknowledged.

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#### TYPICAL PROTOCOLS OF GROUP I

(Observations recorded in these protocols are those made at the beginning of the period of observation, shortly before therapy was begun, and afterward. The abbreviations used are as follows: P = Pulse; BP = Blood pressure; RBC = Red blood cells (million per cubic micra); Hgb = Hemoglobin (grams per 100 cc. of blood); Hct = Hematocrit (per cent); MCV = Mean corpuscular volume (cubic micra); MCH = Mean corpuscular hemoglobin (grams); MCHC = Mean corpuscular hemoglobin concentration (grams per cent); Ret = Reticulocytes (per cent); Gas = Gastric secretion (HCl).

*Subject 7 (D. P.): 21 years; para 0*

*December 19, 1934:* Original observations on subject 62 days pregnant. No symptoms, physical examination normal. P 88; BP 116/72. Gastric analysis—Fasting contents: cc. of juice per hour 20; free HCl 56, T.A. 60; 40 minutes after alcohol: free HCl 48, T.A. 58. RBC 4.00; Hgb 13.5; Hemcrt 39.7; MCV 99; MCHgb 34.

*June 7, 1935:* Painful ulcers of the tongue have developed and several of them have coalesced into one which involves most of the dorsum. This large ulcer is shallow, the base is red, and the edges are white. Similar ulcers of the angles of the mouth are present. Vomiting occasionally, and intermittent diarrhea is present. Vibratory sensation is impaired over all the toes. Moderate edema over the tibiae. Complaints of dysphagia, burning in the esophagus, epigastric distress after eating and abdominal cramps. Appetite is very poor. Tires easily. P 88; BP 110/74. EKG: unstable rate. Gastric analysis—Fasting contents: cc. of juice per hour 40; free HCl 54, T.A. 60; 40 minutes after alcohol: free HCl 44, T.A. 50. RBC 2.81; Hgb 11.3; Hemcrt 34.7; Ret 2.1; MCV 124; MCHgb 40; macrocytosis, occasional megalocytes, polychromasia, occasional poikilocytes.

*June 9:* Liver extract, 2 cc., given intramuscularly, to be continued for the remainder of observation. June 13: Ret 3.1. June 18: Ret 5.4. June 22: Ret 0.1.

*June 25:* During the last 17 days has received 2 cc. of liver extract intramuscularly daily. Except for slight continued anorexia is without symptoms and considers herself perfectly well. Ulcers of the tongue and lips are entirely healed, vibratory sense is normal, no edema. P 120; BP 122/88. EKG: unstable rate. Gastric analysis—Fasting contents: cc. of juice per hour 52, free HCl 30, T.A. 36; 40 minutes after alcohol: free HCl 42, T.A. 58. RBC 3.45; Hgb 11.6; Hemcrt 36.7; Ret 0.1; MCV 106; MCHgb 34. The blood is normal in appearance, except for a moderate number of macrocytes. The administration of liver extract is discontinued and brewer's yeast is to be taken orally until delivery.

*July 25:* Delivered of a full term normal male infant weighing 8 pounds and 15 ounces.

*Subject 3 (E. C.): 19 years; para 0*

*January 27, 1935:* Original observations on subject 112 days pregnant. No symptoms; physical examination is negative. P 72; BP 106/76. Gastric analysis—Fasting contents: cc. of juice per hour 60, free HCl 0, T.A. 18; 40 minutes after alcohol: free HCl 12, T.A. 32. RBC 4.74; Hgb 13.2; Hemcrt 41.2; MCV 93; MCHgb 28.

*April 13:* The tongue is smooth and red. Vibratory sense is normal, there is slight edema over the tibiae. Complaints of dysphagia, of marked heartburn and of epigastric distress after eating. Appetite is very poor. Constipation is marked. Intermittent abdominal cramps. Severe paresthesias and cramps in the legs. Tires very easily. P 112; BP 106/68. EKG: unstable rate. Gastric analysis—Fasting contents: cc. of juice per hour 36, free HCl 0, T.A. 6; 40 minutes after alcohol: free HCl 2, T.A. 8; excessive mucus in all specimens. RBC 3.87; Hgb 14.0; Hemcrt 45.3; Ret 2.1; MCV 117; MCHgb 36.2; macrocytosis, polychromasia, occasional poikilocytes. The administration of 21 grams of brewer's yeast daily is begun. April 20: Ret 2.6. May 5: Ret 1.9.

*May 18:* Except for mild heartburn is without symptoms and considers herself perfectly well. The tongue is still redder than normal but the papillae have regenerated. Vibratory sense is normal. No edema. P 84; BP 102/78. Gastric analysis—Fasting contents: cc. of juice per hour 88, free HCl 8, T.A. 12; 40 minutes after alcohol: free HCl 32, T.A. 40; no mucus. RBC 4.65; Hgb 15.6; Hemcrt 47.6; Ret 1.5; MCV 102; MCHgb 34. The blood is normal in appearance except for a moderate number of macrocytes.

*May 29:* Delivered of a full term normal female infant weighing 7 pounds and 2 ounces.

*Subject 1 (C. G.): 22 years; para II*

*February 11, 1935:* Original observations on subject 82 days pregnant. Slight dysphagia, physical examination negative. P 84; BP 106/64. Gastric analysis—Fasting contents: cc. of juice per hour 68, free HCl 16, T.A. 44; 40 minutes after alcohol: free HCl 4, T.A. 18. RBC 3.65; Hgb 13.0; Hemcrt 38.0; MCV 104; MCHgb 36.

*April 15:* The tongue is smooth, red and pain-



ful. History of alternating constipation and diarrhea. Vibratory sense is lost over the great toes and impaired elsewhere in the lower extremities. Edema is moderate over the tibiae. Complains of marked dysphagia and heartburn. The appetite is poor and epigastric distress occurs after eating. Severe paresthesias and cramps in the lower extremities. Tires easily. P 100; BP 114/84. Gastric analysis—Fasting contents: cc. of juice per hour 20, free HCl 38, T.A. 58; 40 minutes after alcohol: free HCl 26, T.A. 36. RBC 3.01; Hgb 11.3; Hemcrt 37.4; Ret 4.0; MCV 124; MCHgb 38; macrocytosis, frequent megalocytes, occasional poikilocytes, moderate polychromasia. The administration of 21 grams

of brewer's yeast daily is begun. April 22: Ret 5.4. April 27: Ret 5.1. May 4: Ret 2.8. May 15: Ret 2.5.

*May 20:* Slight anorexia and occasional diarrhea, otherwise free of symptoms. The tongue is normal in appearance. Vibratory sense is normal. Slight edema. P 88; BP 116/78. Gastric analysis—Fasting contents: cc. of juice per hour 52, free HCl 8, T.A. 16; 40 minutes after alcohol: free HCl 28, T.A. 38. RBC 3.36; Hgb 11.7; Hemcrt 38.6; MCV 115; MCHgb 35. There are occasional macrocytes, poikilocytes and some polychromatic cells.

*May 29:* Delivered of a full term normal female infant weighing 7 pounds and 3 ounces.

# CHRONAXIMETRIC EXAMINATIONS IN B AVITAMINOSIS DURING PREGNANCY

By F. H. LEWY

*(From the Gastro-Intestinal Section of the Medical Clinic, Hospital of the University of Pennsylvania, Philadelphia)*

(Received for publication February 26, 1937)

In 1929 Lewy and Weisz (1) stated that persons having contact with lead exhibited an increase in the excitability of the nerves and muscles which could be demonstrated chronaximetrically several months before clinical symptoms appeared. That this method might prove valuable as a means of detecting preclinical evidence of peripheral neuritis due to vitamin B deficiency was suggested in 1934 when Lane and Lewy (2) encountered chronaximetric changes in a group of factory workers taking a vitamin B deficient diet but in whom clinical evidence of vitamin B deficiency had not yet developed. To test further the value of this method in demonstrating early vitamin B deficiency, repeated chronaximetric examinations were made upon the group of pregnant women described by Elsom (3) in the preceding paper. Examinations were carried out early in the observation of these subjects, after addition to the diet of yeast or of liver extract and following delivery. The results of these examinations are given in the present communication.

## METHODS OF STUDY

Chronaximetric examinations were made at each visit. Unless otherwise stated the radial nerve was used. The tests were made independently of the other procedures carried out so that not until after the delivery of each subject was this examiner aware of the associated observations made by Elsom (3).

The theory and technique of chronaximetric measurement have been described in detail elsewhere (4). Suffice it to state here that chronaxie is a measurement of the time a current of doubled threshold value must pass through a tissue in order to elicit the first muscle twitch or the first sensation. In the present examinations the time was determined with currents not only double the threshold value, but also with five or six more values.

From the information so obtained, a strength duration curve could be constructed characteristic of the nerve irritability. It is believed that repeated examinations of this type are necessary to obtain an accurate picture of the chronaxie for each individual.

## RESULTS

In Table I are given the results of chronaximetric examinations in eight subjects as compared with clinical and blood changes observed in these persons at various times throughout the period of observation.

Subject 1 is an example of the fact that the chronaxie may become abnormal before clinical evidence of vitamin B deficiency had been detected. This was indicated by overexcitability of the radial nerve. Two weeks after the administration of brewer's yeast the chronaxie again became normal at the same time that clinical evidence of deficiency disappeared. Following delivery, this patient failed to continue the prescribed yeast and, when examined three weeks later, the radial nerve was again found overexcitable although no clinical evidence of deficiency was as yet manifest.

In Subject 8 likewise chronaximetric changes preceded the appearance of clinical evidence of deficiency. In this individual both clinical and hematological changes were more extensive than in Subject 1. This severity was reflected in the chronaximetric observations by a change from overexcitability of the radial nerve to a condition of underexcitability, a phenomenon which is known to occur in more advanced stages of peripheral neuritis. Following the inauguration of vitamin B therapy gradual return toward normal was noted in all types of observations so that at the time of delivery all examinations were negative.

Observations similar to those described above were made in each of the eight women studied.

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Observations similar to those described above were made in each of the eight women studied.

Correlation between clinical, hematological and chronaximetric studies was close in each instance. However, the nerve once degenerated, i.e., under-excitable, a longer time was required for its restitution than for the blood regeneration.

#### COMMENT

The present investigations have shown that electric irritability of the peripheral nerves occurred even before clinical changes were noted in some of eight pregnant women taking diets which proved to be deficient in vitamin B. Upon the addition of yeast or of liver extract to the diet the neuropathy, as detected by chronaximetric examination, improved concomitant with clinical improvement. There was correlation also between the severity of clinical evidence of deficiency and the degree of chronaximetric change in the peripheral nerves examined.

It is considered wise to emphasize the desirability of determining the whole strength duration

curve for nerve or muscle at each examination. This is particularly important in detecting the first stages of nerve degeneration and the last stages of regeneration. Two further points are worthy of mention. We examined the chronaxie of the motor system as we wished to be independent of the subjective statements of the patients. There is, however, little doubt that, in these patients as in lead poisoning, the chronaxie of the sensory system should give a still earlier and finer indication of the beginning abnormality (Altenburger (5)). And finally, although in the present investigation the radial system was selected as the site of examination to avoid the objections which might arise from a possible pressure on the sciatic nerve, it is believed that the peroneal or the sciatic systems are preferable sites for examination since subjective and objective symptoms in our patients were prominent in the legs and very infrequent in the arms. The

TABLE I  
*Results of chronaximetric examinations*

Case number	Date	Diet	Changes in		Sensory disturbances		Chronaxie			Excitability†
			Blood	Clinical manifestations	Vibratory sense	Other signs and symptoms	Volt	$\sigma^*$	vc†	
1.	February 9	Without added vitamin B	0	0	Normal	0	80	0.76	7.8	Normal
	March 2	Without added vitamin B	0	0	Normal	0	80	0.36	5.4	Increased
	March 16	Without added vitamin B	0	0	Normal	Radial nerves tender to pressure. Hyperpathy in area of cutaneous femoral lateral nerve and of 2d sacral root	65	0.28	4.5	Increased
	March 25	Without added vitamin B	+	+	Normal	Radial nerves tender to pressure. Hyperpathy in area of cutaneous femoral lateral nerve and of 2d sacral root				
	April 13	Supplemented with yeast since April 13	+	+	Diminished	Radial nerves tender to pressure. Hyperpathy in area of cutaneous femoral lateral nerve and of 2d sacral root	70	0.40	5.3	Increased
	April 27	Supplemented with yeast	0	0	Slightly diminished	0	90	0.48	6.6	Normal
	May 20	After delivery without added vitamin B	0	0	Normal	0	100	0.24	4.7	Increased
2.	May 4	Without added vitamin B	++	+	++	0	110	0.72	8.9	Superior limit of normal
	June 26	Supplemented with yeast	0	0	0	0	75	0.68	6.9	Normal
3.	April 13	Without added vitamin B	+++	+++	++	Hyperpathy in area of right 2d sacral root; numbness of left arm	80	1.20	9.8	Decreased
	May 27	Supplemented with yeast since April 14	0	0	0	0	Discontinuous curve		6.5 12.5	Normal Decreased
	June 24	After delivery without added vitamin B				Numbness and weakness of left arm	105	2.00	14.5	Decreased

TABLE 1—Continued

Case number	Date	Diet	Changes in		Sensory disturbances		Chronaxie			Excitability†
			Blood	Clinical manifestations	Vibratory sense	Other signs and symptoms	Volt	$\sigma^*$	vc‡	
4.	April 6	Without added vitamin B	0	0	0	0	85	0.40	5.3	Increased
	May 4	Without added vitamin B	+++	++	+	0	90	0.40	5.2	Increased
	May 29	Supplemented with yeast since May 5	++	0	0	Left leg sleepy. Left peroneal nerve tender to pressure	Discontinuous curve	8.8	10.0	Top normal Decreased
	June 17	Supplemented with yeast since May 5	+	0	0	0	80	0.80	6.6	Normal
5.	April 24	Without added vitamin B	+++	+++	++	0	60	0.60	6.0	Inferior limit of normal
	May 11	Supplemented with yeast	+	+	0					
	June 12	After delivery				0	90	0.60	7.3	Normal
6.	April 15	Without added vitamin B	+++	+	+	0	60	1.87	10.5	Decreased
		Supplemented with yeast	0	0	0					
7.	June 6	Without added vitamin B	+++	+++	+	Hyperpathy in area of 2d sacral root	60	4.80	17.0	Decreased
	June 26	Supplemented with yeast	+	0	0	0	Discontinuous curve	7.7	14.5	Normal Decreased
8.	February 6	Without added vitamin B	0	0	Normal	0	75	0.68	7.1	Normal
	March 9	Without added vitamin B	0	0	Normal	0	80	0.36	5.4	Increased
	March 20	Without added vitamin B	0	0	Normal	Hyperpathy in area of 2d sacral root	50	0.48	4.9	Increased
	April 13	Without added vitamin B	+++	+	Normal					
	April 27	Without added vitamin B	+++	+	Normal	Plus numbness in left leg	90	0.84	9.3	Decreased
	May 4	Supplemented with yeast	+++	+	Slightly diminished	Plus numbness in left leg				
	May 27	Supplemented with yeast	++	0	Normal	Less discomfort	Discontinuous curve	7.0	10.0	Normal Decreased
	June 6	Supplemented with yeast	+	0	Normal	Less discomfort	75	0.42	5.5	Increased
	June 17	Supplemented with yeast	0	0	Normal	0	80	0.80	6.7	Normal

\*  $\sigma = 1/1000$  second; normal range about 0.44 to 0.8 $\sigma$ .

† vc = Vertex coordinate the normal of which is between 6 and 9 for the examined nerve muscle apparatus.

‡ Examined at the *muscularis extensor digitorum communis* and on the radial nerve.

same affinity of a noxious agent for the inferior extremities has been noted previously (Tietze (6)) in the neuritis secondary to exposure to carbon disulphide or to methyl bromide.

#### SUMMARY

Chronaximetric changes were observed in the radial nerves of a group of pregnant women taking diets which proved to be deficient in vitamin B. These changes often preceded clinical and hematological evidence of vitamin B deficiency.

The degree of peripheral nerve change, as indicated by chronaximetric examination, coincided with the severity of clinical manifestations of deficiency.

Improvement following vitamin B therapy was observed chronaximetrically at the time that clinical improvement was recorded.

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Correlation between clinical, hematological and chronaximetric studies was close in each instance. However, the nerve once degenerated, i.e., under-excitable, a longer time was required for its restitution than for the blood regeneration.

#### COMMENT

The present investigations have shown that electric irritability of the peripheral nerves occurred even before clinical changes were noted in some of eight pregnant women taking diets which proved to be deficient in vitamin B. Upon the addition of yeast or of liver extract to the diet the neuropathy, as detected by chronaximetric examination, improved concomitant with clinical improvement. There was correlation also between the severity of clinical evidence of deficiency and the degree of chronaximetric change in the peripheral nerves examined.

It is considered wise to emphasize the desirability of determining the whole strength duration

curve for nerve or muscle at each examination. This is particularly important in detecting the first stages of nerve degeneration and the last stages of regeneration. Two further points are worthy of mention. We examined the chronaxie of the motor system as we wished to be independent of the subjective statements of the patients. There is, however, little doubt that, in these patients as in lead poisoning, the chronaxie of the sensory system should give a still earlier and finer indication of the beginning abnormality (Altenburger (5)). And finally, although in the present investigation the radial system was selected as the site of examination to avoid the objections which might arise from a possible pressure on the sciatic nerve, it is believed that the peroneal or the sciatic systems are preferable sites for examination since subjective and objective symptoms in our patients were prominent in the legs and very infrequent in the arms. The

TABLE I  
*Results of chronaximetric examinations*

Case number	Date	Diet	Changes in		Sensory disturbances		Chronaxie			Excitability†
			Blood	Clinical manifestations	Vibratory sense	Other signs and symptoms	Volt	σ*	vct†	
1.	February 9	Without added vitamin B	0	0	Normal	0	80	0.76	7.8	Normal
	March 2	Without added vitamin B	0	0	Normal	0	80	0.36	5.4	Increased
	March 16	Without added vitamin B	0	0	Normal	Radial nerves tender to pressure. Hyperpathy in area of cutaneous femoral lateral nerve and of 2d sacral root	65	0.28	4.5	Increased
	March 25	Without added vitamin B	+	+	Normal	Radial nerves tender to pressure. Hyperpathy in area of cutaneous femoral lateral nerve and of 2d sacral root				
	April 13	Supplemented with yeast since April 13	+	+	Diminished	Radial nerves tender to pressure. Hyperpathy in area of cutaneous femoral lateral nerve and of 2d sacral root	70	0.40	5.3	Increased
	April 27	Supplemented with yeast	0	0	Slightly diminished	0	90	0.48	6.6	Normal
	May 20	After delivery without added vitamin B	0	0	Normal	0	100	0.24	4.7	Increased
2.	May 4	Without added vitamin B	+++	+	++	0	110	0.72	8.9	Superior limit of normal
	June 26	Supplemented with yeast	0	0	0	0	75	0.68	6.9	Normal
3.	April 13	Without added vitamin B	+++	+++	++	Hyperpathy in area of right 2d sacral root; numbness of left arm	80	1.20	9.8	Decreased
	May 27	Supplemented with yeast since April 14	0	0	0	0	Discontinuous curve	6.5 12.5		Normal Decreased
	June 24	After delivery without added vitamin B				Numbness and weakness of left arm	105	2.00	14.5	Decreased

TABLE I—Continued

Case number	Date	Diet	Changes in		Sensory disturbances		Chronaxie			Excitability†
			Blood	Clinical manifestations	Vibratory sense	Other signs and symptoms	Volt	$\sigma^*$	vc†	
4.	April 6	Without added vitamin B	0	0	0	0	85	0.40	5.3	Increased
	May 4	Without added vitamin B	+++	++	+	0	90	0.40	5.2	Increased
	May 29	Supplemented with yeast since May 5	++	0	0	Left leg sleepy. Left peroneal nerve tender to pressure	Discontinuous curve		8.8 10.0	Top normal Decreased
	June 17	Supplemented with yeast since May 5	+	0	0	0	80	0.80	6.6	Normal
5.	April 24	Without added vitamin B	+++	+++	++	0	60	0.60	6.0	Inferior limit of normal
	May 11	Supplemented with yeast	+	+	0					
	June 12	After delivery				0	90	0.60	7.3	Normal
6.	April 15	Without added vitamin B	+++	+	+	0	60	1.87	10.5	Decreased
		Supplemented with yeast	0	0	0					
7.	June 6	Without added vitamin B	+++	+++	+	Hyperpathy in area of 2d sacral root	60	4.80	17.0	Decreased
	June 26	Supplemented with yeast	+	0	0	0	Discontinuous curve		7.7 14.5	Normal Decreased
8.	February 6	Without added vitamin B	0	0	Normal	0	75	0.68	7.1	Normal
	March 9	Without added vitamin B	0	0	Normal	0	80	0.36	5.4	Increased
	March 20	Without added vitamin B	0	0	Normal	Hyperpathy in area of 2d sacral root	50	0.48	4.9	Increased
	April 13	Without added vitamin B	+++	+	Normal					
	April 27	Without added vitamin B	+++	+	Normal	Plus numbness in left leg	90	0.84	9.3	Decreased
	May 4	Supplemented with yeast	+++	+	Slightly diminished	Plus numbness in left leg				
	May 27	Supplemented with yeast	++	0	Normal	Less discomfort	Discontinuous curve		7.0 10.0	Normal Decreased
	June 6	Supplemented with yeast	+	0	Normal	Less discomfort	75	0.42	5.5	Increased
	June 17	Supplemented with yeast	0	0	Normal	0	80	0.80	6.7	Normal

\*  $\sigma = 1/1000$  second; normal range about 0.44 to 0.8 $\sigma$ .

† vc = Vertex coordinate the normal of which is between 6 and 9 for the examined nerve muscle apparatus.

‡ Examined at the *muscularis extensor digitorum communis* and on the radial nerve.

same affinity of a noxious agent for the inferior extremities has been noted previously (Tietze (6)) in the neuritis secondary to exposure to carbon disulphide or to methyl bromide.

#### SUMMARY

Chronaximetric changes were observed in the radial nerves of a group of pregnant women taking diets which proved to be deficient in vitamin B. These changes often preceded clinical and hematological evidence of vitamin B deficiency.

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# THE EFFECT OF HEATING WITH ALKALI ON THE CALORIGENIC ACTIVITY OF DESICCATED THYROID AND OF THYROXINE<sup>1</sup>

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Since thyroxine is usually prepared for intravenous administration by heating with alkali, it seemed desirable, for purposes of comparison, to observe the effect of this procedure on desiccated thyroid. It became apparent at once that it destroyed most of the gland's activity, whereas thyroxine was unaffected by the same treatment (1, 2). This finding took on added interest because heating with alkali is an important step in obtaining thyroxine from the gland.

It was later noted that Roos (3), on the basis of reduction in the size of goiter in man, and Cameron and Carmichael (4), on the basis of the rate of growth and hypertrophy of organs in rats, had reported that heating with potassium and sodium hydroxide destroys a large part of the activity of iodothyron and iodothyroglobulin, respectively. Their methods of assay were, of course, unreliable. Oswald (5) noted the instability of the active thyroid protein and avoided heat when using alkaline hydrolysis. Kendall and Simonsen (6) did employ heat in extracting thyroxine and were sometimes unable to isolate any from desiccated glands which possessed physiological activity before alkaline hydrolysis. In contrast with these observations, Leland and Foster (7) have found that after heating with 2 N sodium hydroxide for eighteen hours, eighty-five per cent of the iodine combined as thyroxine can be extracted with butyl alcohol, suggesting that little if any destruction has occurred.

## METHOD

The total calorigenic response to the oral administration of a certain dose of desiccated thyroid<sup>3</sup> or of thyroxine<sup>4</sup> was observed in patients

with myxedema. (The standard dose contained 6.5 mgm. of iodine, the amount in 10 mgm. of thyroxine.) Then material from the same lot was heated for different lengths of time with approximately normal sodium hydroxide<sup>5</sup> (Table I), usually in the proportion of 5 cc. of alkali to 6.5 mgm. of iodine. The effects of the same procedure without heating, of heating with a weaker solution of alkali, and of heating with distilled water were also observed. The heating was carried out in a small beaker on a water bath. Although the solution was brought up to volume at frequent intervals, some variation in the concentration of alkali occurred. After suitable preparation, the material was diluted with a total of from 500 to 800 cc. of distilled water and administered slowly by mouth over a period of about two hours.

In five of eight patients in whom the effect of heating desiccated thyroid with alkali was observed, the heated and unheated doses contained the same amounts of iodine; in one, the heated dose contained only half as much and in two, twice as much. In two of the patients (Mrs. M. M., Figure 2 and Mrs. M. K., Figure 3) in whom the amounts of iodine in the heated and unheated doses were different, data have been included on the effects of another lot of desiccated thyroid which was given in doses containing more nearly the same amount of iodine as the heated doses. These additional data have been recorded because it has been found essential to compare doses on the basis of similar amounts of iodine, owing to some diminution in effect per unit of iodine with increasing doses (2).

atories. Four lots were used—three lots of hog thyroid and one of the dried gland from a patient with exophthalmic goiter.

<sup>3</sup> The synthetic thyroxine of Hoffmann-La Roche.

<sup>5</sup> The solution actually used was 1.14 N sodium hydroxide.

<sup>1</sup> Brief references to this work have been previously published (1, 2).

<sup>2</sup> Squibb Research Fellow.

<sup>3</sup> The desiccated thyroid, unless otherwise noted, was in the form of powder which had been defatted with benzene and was kindly supplied by Dr. Klein of the Wilson Lab-

TABLE II  
Summary of results

Medication	Method of administration	Iodine content*	Total number of patients	Total number of administrations	Number of patients in this series	Number of administrations in this series	Average basal metabolic rate before administration	Average level to which basal metabolic rate rose	Average change in basal metabolic rate	Loss of activity as a result of heating	Average number of excess calories produced	Loss of activity as a result of heating	Change in terms of response to intravenous injection of 10 mgm. thyroxine (6.5 mgm. iodine) in alkaline solution	
													On basis of increase in basal metabolic rate	On basis of excess calories produced
		mgm.					per cent normal	per cent normal	points	points per cent	points per cent	calories per cent	per cent	per cent
Thyroxine in alkaline solution (synthetic and Squibb's)	Intravenously	6.5	6	8	4	6	-37	-5	32		15,520		100	100
Thyroxine in alkaline solution (synthetic)	By mouth	6.5	6	6	5	5	-34	-11	23		11,625		72	75
Patients who also received thyroxine in alkaline solution heated	By mouth	6.5	2	2	2	2	-34	-8	26		16,850		81	109
Thyroxine in alkaline solution (synthetic) heated	By mouth	6.5	2	2	2	2	-34	-7	27	0	15,440	8	84	99
Desiccated hog thyroid (all lots) suspended in distilled water	By mouth	6.5	16	18	11	13	-36	-15	21		8,680		66	56
Patients who also received desiccated hog thyroid in alkaline solution without heating	By mouth	6.5	2	3	2	3	-42	-23	19		7,260		59	47
Patients who also received desiccated hog thyroid heated with alkali	By mouth	6.5	4	5	4	5	-37	-17	20		6,760		63	44
Patients who also received desiccated hog thyroid heated with distilled water	By mouth	6.5	2	2	2	2	-38	-18	20		7,230		63	47
In alkali—not heated	By mouth	6.5	2	3	2	3	-40	-23	17		6,090		53	39
In alkali—heated	By mouth	6.5	4	4	4	4	-37	-29	8	60	1,360	80	25	9
Suspended in distilled water—heated	By mouth	6.5	2	2	2	2	-38	-17	21	0	7,470	0	66	48
Desiccated exophthalmic goiter thyroid (J. W.) suspended in distilled water	By mouth	6.5	4	4	4	4	-33	-7	26		11,950		81	77
Patients who also received desiccated exophthalmic goiter thyroid heated with alkali	By mouth	6.5	3	3	3	3	-34	-5	29		14,875		91	96
In alkali—heated	By mouth	6.5	3	3	3	3	-33	-23	10	66	1,825	88	31	12

\* For purposes of comparison, all doses were calculated in terms of 6.5 mgm. of iodine.

† In one patient the dose contained 4.9 mgm. of iodine.

TABLE III

Medication	Method of administration	Iodine content	Number of patients	Number of administrations	Average basal metabolic rate before administration	Average level to which basal metabolic rate rose	Average change in basal metabolic rate	Loss of activity as a result of heating	Average number of excess calories produced	Loss of activity as a result of heating	Change in terms of response to intravenous injection of 10 mgm. of thyroxine in alkaline solution	
											On basis of increase in basal metabolic rate	On basis of excess calories produced
											per cent	per cent
Thyroxine in alkaline solution (synthetic)	Intravenously	6.5	3	5	-32	-4	28	10,060	100	100	100	100
Patients who also received desiccated thyroid heated with alkali *												
Thyroxine in alkaline solution (synthetic)	By mouth	6.5	4	4	-32	-9	23	10,340	82	103		
Patients who also received desiccated thyroid heated with alkali	By mouth	6.5	3	3	-34	-13	21	7,675	75	76		
Desiccated thyroid suspended in distilled water	By mouth	6.5	4	4	-32	-12	20	6,070	71	60		
Patients who also received desiccated thyroid heated with alkali	By mouth	6.5	3	3	-33	-13	20	6,185	71	61		
Desiccated thyroid in alkali heated	By mouth	6.5	4	4	-32	-24	8	1,210	29	12		
Patients who received thyroxine in alkaline solution by mouth	By mouth	6.5	3	3	-33	-25	8	1,425	29	14		
Patients who received thyroxine intravenously												

\* For more complete data on these patients, see a previous communication (9).

Excess calories were calculated by a method previously described (8, 9).

#### DATA

##### *Effect of heating desiccated thyroid with alkali*

The data are recorded in Tables I, II and III and in Figures 1 to 8. The results may be summarized as follows.

1. After heating with approximately normal sodium hydroxide for from one to seven hours, desiccated thyroid loses about three-fifths of its calorigenic activity, on the basis of the number of points increase in metabolism; and about four-fifths of it, on the basis of the number of extra calories produced (Table II).

2. This loss of activity appears to be about as great at the end of one hour as at the end of seven hours (Miss R. G., Figure 5, compared with Mr. G. H., Figure 1, and Mrs. M. J., Figure 6).

3. The strength of the alkali appears to be important. In the one instance in which heating

with alkali did not cause loss of activity (Mrs. M. S., Figure 8) the alkali used was 0.1 N instead of 1 N.

4. Allowing the dried gland to stand in 1 N sodium hydroxide without heating did not cause a significant loss of activity (Mr. G. H., Figure 1; Mrs. M. M., Figure 2; Mrs. M. K., Figure 3; Mrs. A. R., Figure 4; Mrs. M. W., Figure 7).

5. The relative loss of activity in the exophthalmic goiter gland as a result of heating with alkali appeared to be about the same as in the hog thyroid (Table II; and Figures 5 to 8 compared with Figures 1 to 4).

Repeated administrations of the same dose of desiccated thyroid or of thyroxine to the same patient produce about the same calorigenic response. Therefore, the slight response to thyroid which had been heated with alkali cannot be explained on the basis of the development of a tolerance.

The question arises as to whether the calorigenic effects of different preparations should be compared on the basis of the amount of increase in the basal metabolism or on the number of extra calories produced, the latter showing approximately a twenty per cent greater loss of activity on the average than the former. The number of extra calories is calculated from a curve denoting the change in metabolism from the time a given preparation is administered until its effect has completely disappeared. There are reasons for believing that the total response is important in comparing the action of different compounds. For example, it would be unfair to compare the effects of dinitrophenol and thyroxine on the basis of the amount of increase in basal metabolism, because the effect of a single dose of dinitrophenol lasts for only three or four days, while that of a single dose of thyroxine may last for as long as eighty days (2).

##### *Effect of heating desiccated thyroid with distilled water*

In contrast with the marked loss of calorigenic activity produced by heating desiccated thyroid with alkali, it may be seen from Tables I and II and Figures 9 and 10 that heating desiccated thyroid with distilled water for three hours and nine hours respectively produced no loss of activity.

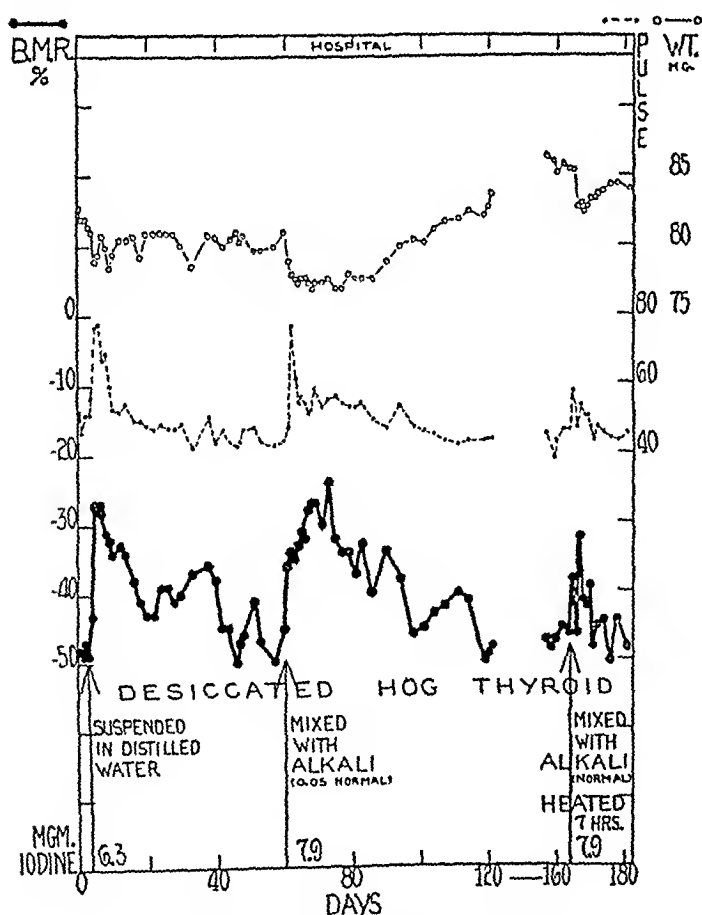


FIG. 1. MR. G. H. COMPARATIVE EFFECTS OF THE ORAL ADMINISTRATION OF DESICCATED HOG THYROID SUSPENDED IN DISTILLED WATER, AND MIXED WITH ALKALI, WITH AND WITHOUT HEATING

# EFFECT OF HEATING WITH ALKALI ON THYROID

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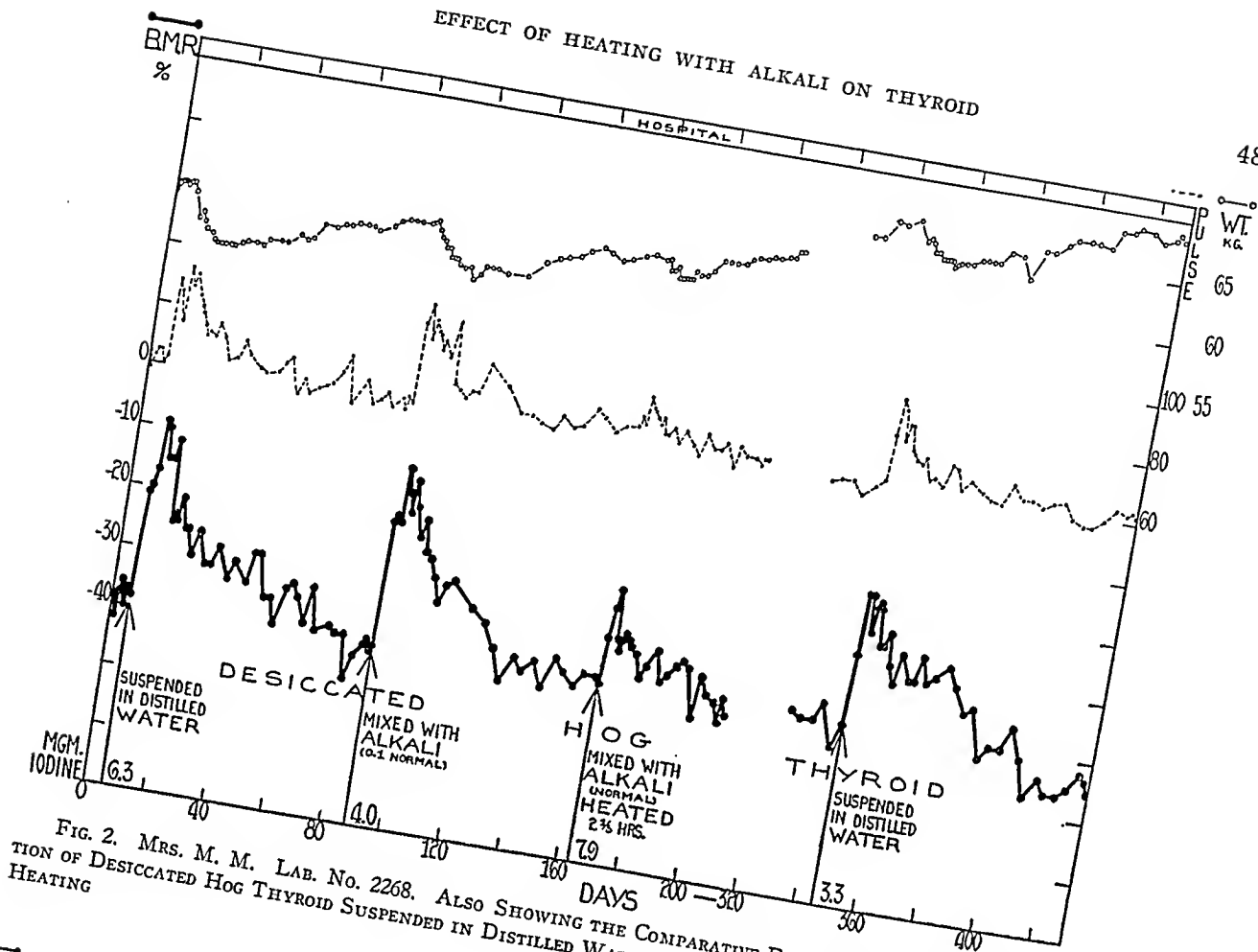


FIG. 2. Mrs. M. M. LAB. No. 2268. ALSO SHOWING THE COMPARATIVE EFFECTS OF THE ORAL ADMINISTRATION OF DESICCATED HOG THYROID SUSPENDED IN DISTILLED WATER, AND MIXED WITH ALKALI, WITH AND WITHOUT HEATING

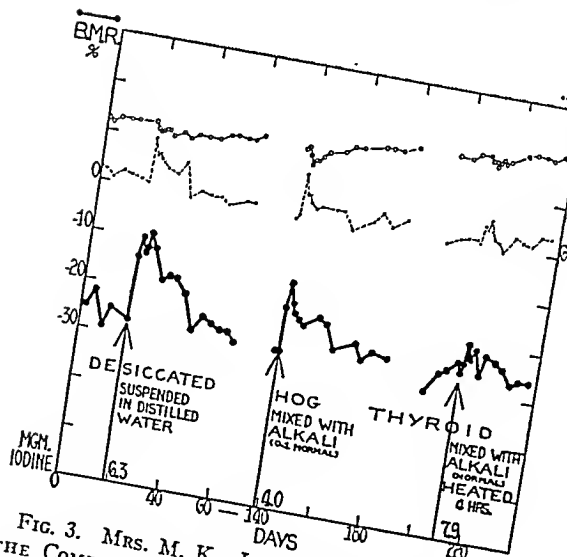


FIG. 3. Mrs. M. K. LAB. No. 2040. ALSO SHOWING THE COMPARATIVE EFFECTS OF THE ORAL ADMINISTRATION OF DESICCATED HOG THYROID SUSPENDED IN DISTILLED WATER, AND MIXED WITH ALKALI, WITH AND WITHOUT HEATING

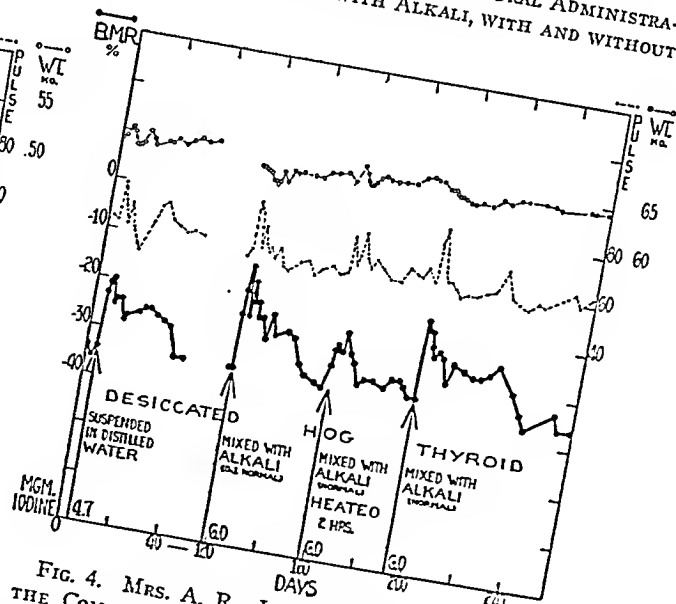


FIG. 4. Mrs. A. R. LAB. No. 1000. ALSO SHOWING THE COMPARATIVE EFFECTS OF THE ORAL ADMINISTRATION OF DESICCATED HOG THYROID SUSPENDED IN DISTILLED WATER, AND MIXED WITH ALKALI, WITH AND WITHOUT HEATING

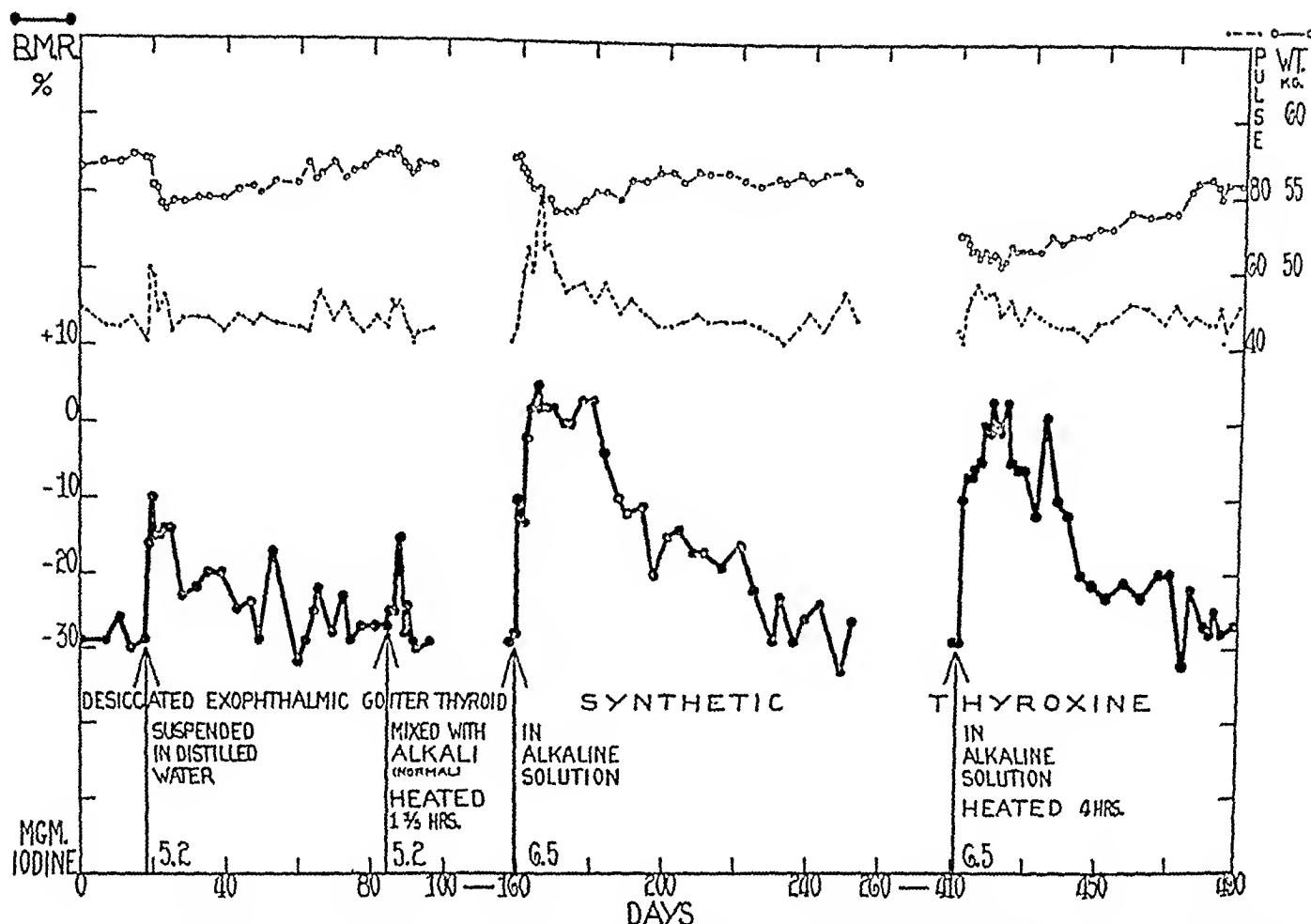


FIG. 5. MISS R. G. LAB. NO. 2933. COMPARATIVE EFFECTS OF THE ORAL ADMINISTRATION OF DESICCATED EXOPHTHALMIC GOITER THYROID SUSPENDED IN DISTILLED WATER AND HEATED WITH ALKALI: ALSO OF THYROXINE IN ALKALINE SOLUTION, WITH AND WITHOUT HEATING

Although more data are desirable on this point, they are sufficient to show that in order to produce the marked loss of activity reported above, the presence of alkali is necessary.

#### *Effect of heating thyroxine with alkali*

It may be seen from Tables I and II and Figures 5 and 11 that thyroxine apparently loses no activity as a result of heating with normal sodium hydroxide for four and four and three-quarter hours respectively.

#### COMMENT

There are at least four possible explanations for these observations.

1. Although only a small portion of the iodine in the thyroid may be present as thyroxine, the activity of thyroxine may be greatly enhanced by the form or combination in which it occurs and one or both of these may be altered by heating with alkali.

2. Only a portion of the calorogenic activity of

desiccated thyroid may be caused by the thyroxine in it and the other iodine compound or compounds in the gland which affect metabolism may be destroyed by heating with alkali.

3. Thyroxine in its natural combination may be more susceptible to destruction by heating with alkali than the free amino-acid.

4. "Thyroxine as 'isolated' may be formed 'as an artefact by the action of the rather drastic method of isolation of the active principle,' Harington (10). (Apparently disproved by Harington and Salter (11).)

Any combination of these various factors may be involved. Thus, heating with alkali may reduce activity both by destroying the natural form or combination of thyroxine and by destroying or reducing the activity of other compounds in the gland which possess activity.

In view of the recent work of Foster, Palmer and Leland (12) on the calorogenic potencies of l- and dl-thyroxine, it is necessary to consider the possibility that the loss of activity produced by

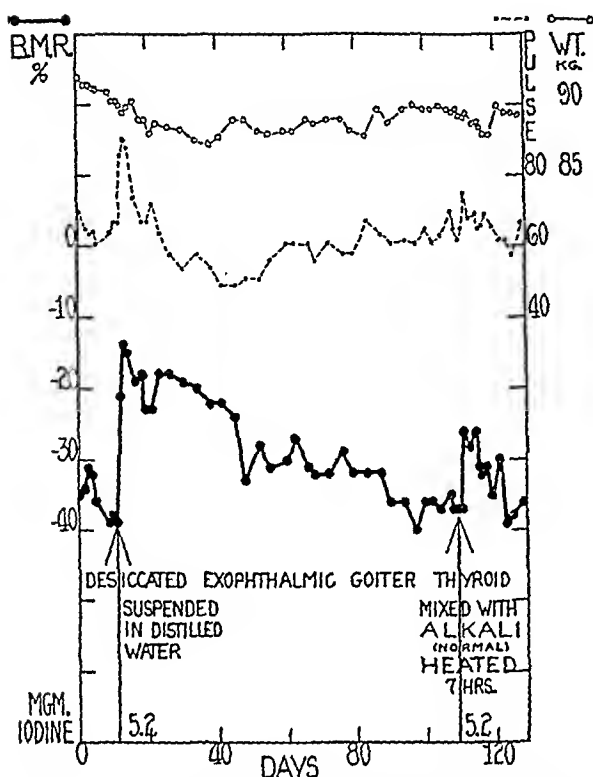


FIG. 6. MRS. M. J. LAB. NO. 3221. COMPARATIVE EFFECTS OF THE ORAL ADMINISTRATION OF DESICCATED EXOPHTHALMIC GOITER THYROID SUSPENDED IN DISTILLED WATER AND HEATED WITH ALKALI

heating desiccated thyroid with alkali may be due to racemization of the naturally occurring l-thyroxine. Using l-thyroxine obtained by the proteolytic digestion of fresh and desiccated thyroid, they found it to be twice as potent as the racemic form in the guinea pig. Gaddum (13), using material obtained by resolution of dl-thyroxine into its two optically active isomers, found l-thyroxine to be from one and one-half to three times as potent as d-thyroxine in the rat. However, too few data are presented to warrant quantitative deductions. Salter, Lerman and Means (14), using material obtained by Harington (15) in the same manner as that supplied to Gaddum, reported the two isomers to possess the same activity in man.

An analysis of our results suggests that the loss of activity was greater than could be accounted for by racemization alone. Assuming that all of the calorigenic potency of desiccated thyroid is due to the thyroxine it contains, complete racemization of the thyroxine should destroy half of

the activity on the basis of the figures of Foster, Palmer and Leland (12). Our smallest figure for loss of activity, namely that based on the number of points the metabolism changed, shows a reduction of 60 per cent as a result of heating with alkali, whereas that based on extra calories shows a reduction of 80 per cent. We did not carry out thyroxine determinations on the hydrolyzed samples which were administered and, therefore, do not know how much was split off. Since the loss of activity was produced with much less alkali and with a much shorter period of hydrolysis than Leland and Foster (7) found necessary to cause maximum separation of thyroxine, it would appear probable that the racemization in our experiments was not complete. If this deduction be correct, we produced greater loss of activity with incomplete racemization than would be accounted for by complete racemization on the basis of the figures of Foster et al. (12).

In considering further the work of Foster, Palmer and Leland, it is of interest to determine

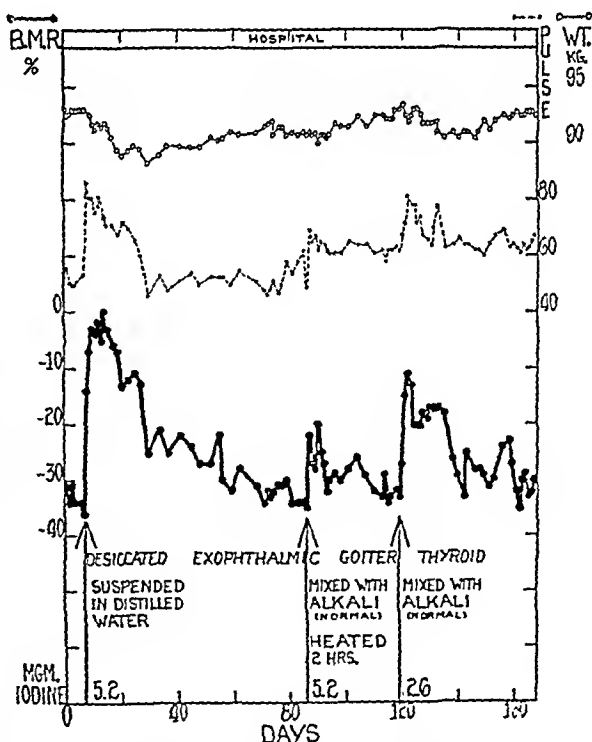


FIG. 7. MRS. M. W. COMPARATIVE EFFECTS OF THE ORAL ADMINISTRATION OF DESICCATED EXOPHTHALMIC GOITER THYROID SUSPENDED IN DISTILLED WATER, AND MIXED WITH ALKALI, WITH AND WITHOUT HEATING



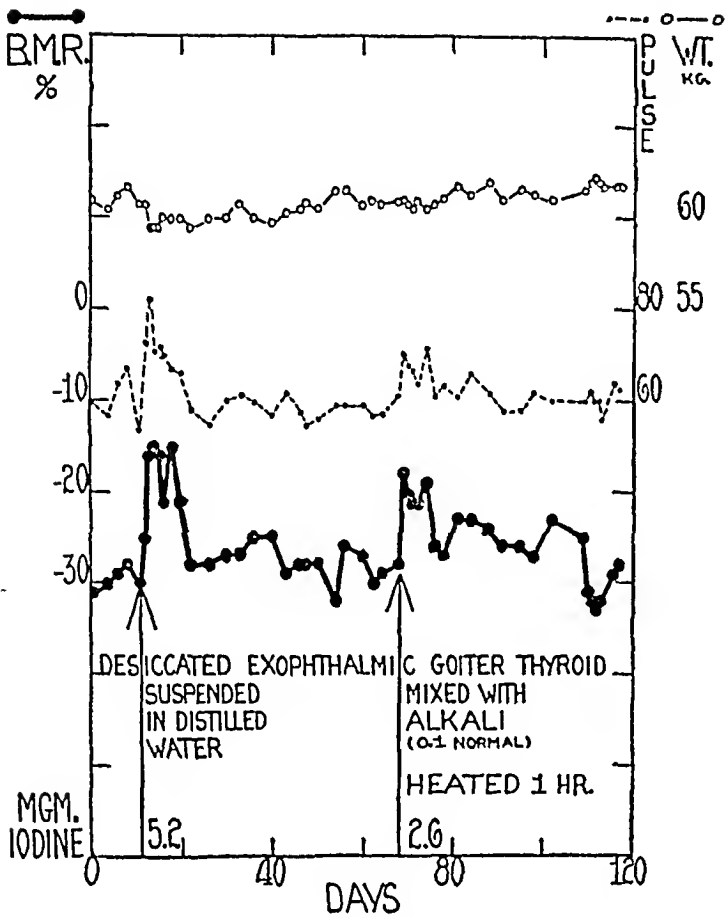


FIG. 8. MRS. M. S. LAB. NO. 3100. COMPARATIVE EFFECTS OF THE ORAL ADMINISTRATION OF DESICCATED EXOPHTHALMIC GOITER THYROID SUSPENDED IN DISTILLED WATER AND HEATED WITH WEAK ALKALI

whether heating with alkali reduces the activity of thyroid to that of the thyroxine which it contains. The calculations which follow are based, of course, on the activity of racemic thyroxine. In four of the patients (Mrs. M. M., Mrs. M. K., Mrs. A. R. and Miss R. G.) the effect of administering thyroxine by mouth in alkaline solution has been compared with that of giving desiccated thyroid suspended in distilled water and desiccated thyroid after heating with alkali (Table III). This comparison in the same patients gives results almost the same as those in Table II, in which two of the oral administrations and three of the intravenous administrations of thyroxine in alkaline solution were in patients who did not receive thyroid which had been heated with alkali. It may be seen that, on the average, for every 6.5 mgm. of iodine administered by mouth in the form of thyroxine in alkaline solution the basal metabolism rose 23 points (from minus 32 per cent to minus 9 per cent) and 10,340 excess calories were produced; whereas for every 6.5 mgm. of iodine given in the

form of desiccated thyroid which had been heated with alkali, the basal metabolism rose 8 points (from minus 32 per cent to minus 24 per cent) and 1,210 excess calories were produced. When the thyroid was given suspended in distilled water the corresponding figures were 20 points and 6,070 excess calories respectively. In other words, per milligram of iodine, thyroxine in alkaline solution produced about three times as much increase in basal metabolism and about eight and one-half times as many excess calories as desiccated thyroid which had been heated with alkali. On the basis of the number of points increase in basal metabolism, heating with alkali reduces the calorogenic activity of desiccated thyroid nearly to the level that would be predicted from the Leland and Foster figures (7) for the percentage of iodine present in the form of thyroxine, assuming that heating with alkali reduces the activity of the dried gland to that of the thyroxine which it con-

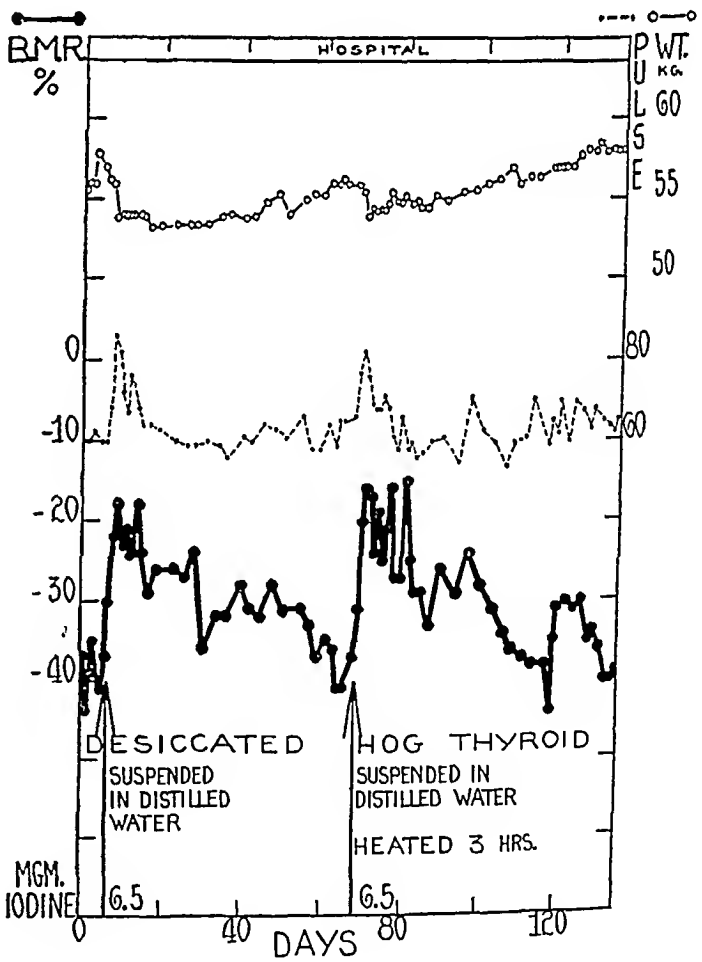


FIG. 9. MISS E. DeL. COMPARATIVE EFFECTS OF THE ORAL ADMINISTRATION OF DESICCATED HOG THYROID SUSPENDED IN DISTILLED WATER, WITH AND WITHOUT HEATING

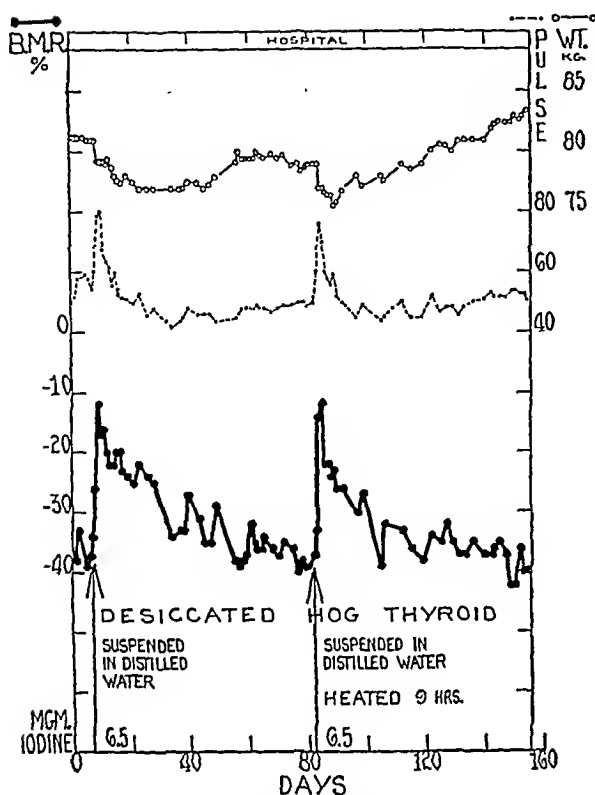


FIG. 10. MRS. B. L. ALSO SHOWING THE COMPARATIVE EFFECTS OF THE ORAL ADMINISTRATION OF DESICCATED HOG THYROID SUSPENDED IN DISTILLED WATER, WITH AND WITHOUT HEATING

tains. On the basis of extra calories, the activity is reduced to a lower level than would be predicted. As already pointed out, this reduction occurs with much less alkali than called for by the Leland and Foster method for extraction of thyroxine from the thyroid. They used 100 cc. of 2 N sodium hydroxide per 1.25 grams of dried gland containing probably from 3 to 4 mgm. of iodine. These observations suggest the possibility that the activity of thyroxine may be enhanced by its natural combination.

The effect of heating desiccated thyroid with alkali has some bearing on the suggestion advanced by Harington and Randall (16) and by Gutman, Benedict and Palmer (17) that, for pharmaceutical purposes, desiccated thyroid should be standardized in terms of thyroxine rather than in terms of total organic iodine. Harington and Randall (16), on the assumption that after four hours hydrolysis with sodium hydroxide the portion of the iodine insoluble in acid represents thy-

roxine iodine, concluded that the iodine in the thyroid is about equally divided between diiodo-tyrosine and thyroxine: while Gutman and his associates (18), using the butyl alcohol extraction method of Leland and Foster (7) (which included a longer period of alkaline hydrolysis), found that about twenty-five per cent was present as thyroxine, although the actual percentage varied in different glands. Using guinea pigs for assay, Palmer and Leland (19) found calorigenic activity proportional to thyroxine rather than to total iodine.

Regardless of the explanation of our observations, it becomes apparent at once that the method used by all investigators for isolation of the active principle from the thyroid, namely hydrolysis with alkali, destroys most of the gland's activity. Indeed, the low yield of crystalline thyroxine from desiccated thyroid has always been one of the most serious handicaps to a systematic study of its properties. From three tons of hog thyroid Kendall (20) obtained thirty-three grams of thyroxine. By another method, Harington (10) was able at one time to obtain a total yield of 0.125 per cent of thyroxine from a preparation of desic-

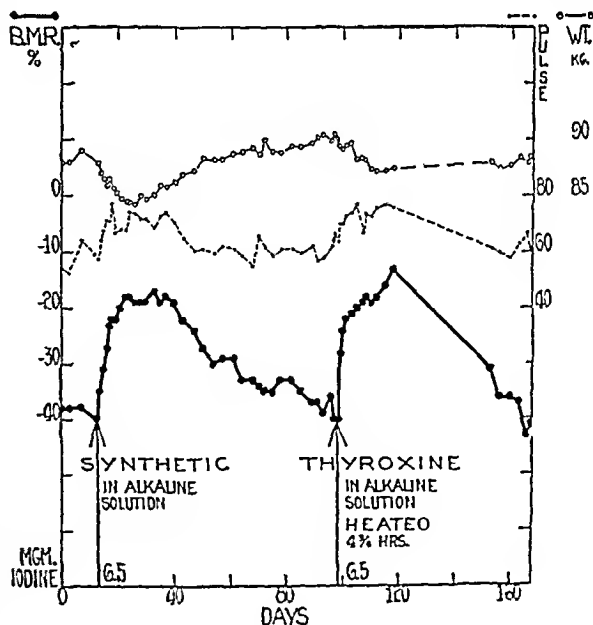


FIG. 11. MRS. C. F. LAD. NO. 2993. COMPARATIVE EFFECTS OF THE ORAL ADMINISTRATION OF THYROID IN ALKALINE SOLUTION, WITH AND WITHOUT HEATING

cated thyroid gland containing 0.5 per cent of iodine. The low yields frequently observed by these two investigators may be attributed to loss or destruction of thyroxine or to the presence of only a small quantity of iodine in the form of thyroxine to begin with.

#### SUMMARY

After heating with approximately normal sodium hydroxide for from one to seven hours, desiccated thyroid loses about three-fifths of its calorigenic activity on the basis of the amount of increase in basal metabolism, and about four-fifths of it on the basis of the number of extra calories produced. The activity of racemic thyroxine is not significantly affected by the same procedure. The effect of desiccated thyroid is not altered when it is heated with distilled water or when it is allowed to stand in normal alkali without heating, showing that a combination of both heat and alkali are necessary to produce loss of activity.

When given by mouth in alkaline solution, thyroxine produces, per milligram of iodine, about three times as much increase in basal metabolism and about eight and one-half times as many extra calories as desiccated thyroid which has been heated with alkali.

These observations show that the procedure common to all methods for isolation of thyroxine from the thyroid (namely, heating with alkali) destroys most of the gland's activity. They have an important bearing on the form in which iodine occurs in the gland and on the methods of standardizing desiccated thyroid.

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# MAGNESIUM METABOLISM IN HEALTH AND DISEASE. I. THE MAGNESIUM AND CALCIUM EXCRETION OF NORMAL INDIVIDUALS, ALSO THE EFFECTS OF MAGNESIUM, CHLORIDE, AND PHOSPHATE IONS

BY DOROTHY M. TIBBETTS AND JOSEPH C. AUB

(From the Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, Boston)

(Received for publication December 22, 1936)

✓ Much attention has been paid in recent years to inorganic salt metabolism. Great advances in our knowledge have been made in regard to the exchange of calcium and sodium, but a surprising ignorance remains in respect to the magnesium exchange. Magnesium is obviously an important constituent of the body for it appears to be universally present, and physiological changes result from magnesium starvation (1). In spite of the fairly close chemical relationship between calcium and magnesium, their relative distribution in body tissues is widely divergent. Roughly, one may state that under normal conditions magnesium tends to be higher than calcium in the cells of soft tissues, while calcium is higher than magnesium in extracellular fluids and in bones. This distribution would make one anticipate that their physiological actions were of different nature, and there is, of course, physiological evidence that sometimes at least their effects are antagonistic. It is, therefore, of interest to see whether the influences which affect calcium metabolism have analogous effects upon magnesium exchange. This is the purpose of the following papers. ✓

From the literature one finds that the concentration of magnesium in body tissues has been studied but little. A good summarizing table of recorded data is given in the recent review by Schmidt and Greenberg (2). The most complete study in man was done by Magnus-Levy in 1910 (3). He studied the inorganic salt content of most of the tissues obtained from a suicide. In the muscles he found magnesium in far greater amounts than calcium, the ratio being approximately 3:1, but in the internal organs there was considerable variation in the ratio, and he found more calcium than magnesium in the skin, thyroid, and lungs. More recent analyses of muscle with improved technic have been reported by Cullen,

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In blood serum and in bones this high concentration of magnesium is not found. In the serum of normal individuals the average value of magnesium in our experience is between 2.4 and 2.8 mgm. per 100 cc. of serum. This agrees quite well with the values from other laboratories. The calcium:magnesium ratio of bone is variously given. In normal dried bone McCrudden (7) found this ratio to be essentially 1:0.005 with a much higher relative magnesium concentration in osteomalacia. Euler and Rydbom (8) give the calcium:magnesium ratio of normal bone ash as 24 parts of calcium to 0.1 part of magnesium and that of rachitic bone as about 21 parts of calcium to 0.6 part of magnesium. Morgulis (9), analyzing the bone ash of various animals, found that the relative concentration of calcium and magnesium differed with the species. The ash of bones of the dog and rabbit he reports as 36 per cent calcium to 0.5 per cent magnesium.

From these concentration ratios of magnesium and calcium, which vary so much in different body tissues, one may obtain approximate evidence of

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From these concentration ratios of magnesium and calcium, which vary so much in different body tissues, one may obtain approximate evidence of

the source of these bases when they are eliminated by the body. If the loss comes entirely from the bone, then the magnesium excretion will be negligible compared with that of calcium; if from the tissues, the magnesium elimination will be the larger. As an example, it was shown by Benedict (10), that in starvation the calcium elimination was approximately four times that of magnesium by weight. This is a ratio unlike that found in the analysis of either bone or tissue. The amount of magnesium would indicate a destruction of the soft tissues but the relatively large amount of calcium eliminated could not be accounted for in this way. In fact, on the basis of the calcium:magnesium ratio, 90 per cent of the calcium must come from the catabolism of bone.

Therefore, it would seem reasonable to assume that changes in magnesium excretion would be slight in cases of bone growth or destruction, but that processes involving tissue catabolism would show more change in magnesium than in calcium excretion.

#### EXPERIMENTAL METHODS AND PROCEDURES

The normal requirements of man for magnesium have not been thoroughly studied. An edible diet which is low in magnesium would have to be high in calcium because the foodstuffs involved would consist largely of milk products and eggs. In most of our observations, we have used a low calcium diet such as we have employed in the past (11). It has a moderate magnesium content of about 220 mgm. per day. This is two or three times the amount excreted during the prolonged starvation in Benedict's study (10), but is below the average (340 mgm.) found by Sherman (5) in 150 American dietaries. We may, therefore, assume that our diets are adequate in their magnesium content though inadequate in calcium. In some observations on the effect of magnesium feeding, we have used ample magnesium and calcium in the diet in order to observe effects on positive as well as on negative balances. All of the diets were potentially neutral and were analyzed in the laboratory for calcium and magnesium.

Our usual metabolic regime as described in a former paper (11) was followed throughout, and the methods of analyses were essentially the same. Calcium determinations were made by the

method of Fiske and Logan as described by Folin (12). In the samples which contained large amounts of magnesium in proportion to the calcium, the determinations were repeated, using the double precipitation method of the same authors (13). As the results were practically identical with those obtained by the former method, we felt satisfied that none of the magnesium was being precipitated with the calcium even though a large amount was present in the sample.

The magnesium determinations were also made by the methods of Fiske and Logan (12). We have used both the colorimetric and the alkalimetric titration methods in these studies, and determinations made on the same sample by the two methods agreed within 3 per cent. When the sample contained a large amount of calcium in proportion to the magnesium, most of the calcium was precipitated as its oxalate salt in a solution acid to methyl red. After removal of this precipitate, the solution was brought to pH 4.8 to 5.0 and the remainder of the calcium oxalate filtered off and the filtrate analyzed for magnesium in the usual way.

Whenever calcium or magnesium was present in large excess, the determinations were carried out independently of each other.

#### EXPERIMENTS

From Table I and the control periods of Tables II and III, it is clear that the magnesium excretion on our type of neutral diet, on the whole, is the same by weight as that of calcium, and that approximately one-third of the total excretion of either base is to be found in the urine. Magnesium is more constant in this regard than is calcium.

Table I also demonstrates that on an intake of 220 mgm. of magnesium daily, some individuals living in the hospital ward will be in negative, some in positive balance. Thus, in 15 periods in five individuals, the average magnesium balance was 50 mgm. per three-day period and in only one case in five was the magnesium balance negative. There were in addition four other normal controls who were medical students pursuing their normal activities, except that all their meals were carefully prepared in the research diet kitchen (A. D. and C. W., Table I; M. L., Table II; and R. L., Table III). They were given a constant



TABLE I  
Normal subjects \*  
(Intake and output in 3-day periods)

Subject *	Period	Magnesium				Calcium			
		Excretion			In- take	Excretion			In- take
		Urine	Feces	Total		Urine	Feces	Total	
		<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
A. N.....	III	.35	.45	.80	.66	.18	.15	.33	.31
Paper XII (20).....	IV	.25	.47	.72	.66	.19	.18	.37	.31
Table I.....	V	.27	.49	.76	.66	.16	.16	.32	.31
B. E.....	II	.27	.49	.76	.74	.10	.47	.57	.37
Paper XII (20).....	III	.24	.20	.44	.72	.13	.16	.29	.34
Table IV.....	IV	.24	.34	.58	.72	.15	.24	.39	.33
	V	.25	.34	.59	.72	.18	.25	.43	.33
D. A.....	XXI	.29	.31	.60	.68	.10	.50	.60	.34
Paper XII (20).....	XXII	.21	.34	.55	.68	.07	.48	.55	.35
Table II.....	XXIII	.30	.31	.61	.68	.07	.45	.52	.35
E. M.....	I	.24	.44	.68	.65	.03	.55	.58	.30
Paper XXII (25) Table I	II	.26	.35	.61	.65	.08	.43	.51	.30
W. N.....	I	.12	.31	.43	.59	.24	.39	.63	.29
Paper XIII (22).....	II	.19	.29	.48	.59	.33	.46	.79	.28
Table I.....	III	.21	.31	.52	.60	.38	.54	.92	.28
Average for 5 subjects...		.25	.36	.61	.66	.16	.36	.52	.32
A. D.....	IX	.30	.47	.77	.95	.49	.44	.93	.31
Paper XXII (25).....	X	.28	.45	.73	.80	.43	.37	.80	.27
Table IV.....	XI	.34	.51	.85	.95	.52	.44	.96	.31
C. W.....	VIII	.34	.51	.85	.95	.44	.65	1.09	.32
Paper XXII (25).....	IX	.29	.60	.89	.95	.38	.52	.90	.32
Table V.....	X	.23	.44	.67	.95	.35	.69	1.04	.32
Average.....		.29	.50	.79	.93	.44	.52	.96	.31

\* Some of the data on these patients have been previously reported. In order to avoid repetition, references are given to the proper paper in the "Studies of Calcium and Phosphorus Metabolism" series (20, 22, 25, 26) where figures on titratable acidity, ammonia, nitrogen, calcium, phosphorus, total base, chloride, and sulphate are recorded. The initials used for each subject are the same as in the previous publications. In these observations on magnesium, transitional periods have not been studied. The period numbers in this and in the previous texts correspond with each other.

diet higher in magnesium content, and two of them (M. L. and R. L.) also had double the usual amount of calcium in the food. On these diets, each of the four subjects showed a positive magnesium balance in the control periods. These observations indicate that an essential magnesium balance with hospital patients is obtained with approximately 220 mgm. intake per day, and that magnesium storage in active subjects may be obtained with 300 mgm. per day. These may not be minimal values. This adequacy of magnesium in the food may obscure positive findings in control periods, but should not interfere with interpretations during the shifting conditions of a prolonged experiment.

#### *The addition of magnesium to the diet*

While on this adequate magnesium diet, two of our normal subjects (M. L. and R. L.) were given 8 to 10 grams of magnesium lactate a day for twelve days. During this time the excretion of magnesium was naturally increased as may be seen in Tables II and III. This increase is found in both urine and feces though, as with calcium, the greater amount of the increase is in the feces, except that in R. L. (Table III) the usual urine: feces ratio is fairly closely maintained. It is clear then that magnesium lactate can be absorbed. Its excretion, like that of calcium, is not rapid for the levels remain elevated in the first control periods following ingestion. It is inter-



esting, however, that in one of these young men (R. L.) the increased ingestion of magnesium resulted in a negative balance, while in the case of the other subject an average of over 200 mgm. per period was retained. Carswell and Winter (14) on feeding magnesium lactate to two normal men found a greater retention of magnesium in one case than in the other, but both stored more than did our subjects. From clinical experience with magnesium sulphate, one might expect some such variation in the absorption of magnesium from the gastro-intestinal tract.

Mendel and Benedict (15) first linked together magnesium and calcium excretion. They showed that parenteral injections of salts of either base increased the urinary excretion of both bases. The effect of oral administration has not been so clearly demonstrated, and the literature on the subject gives conflicting data (16, 17, 18). Carswell and Winter (14) and Barbour and Winter (19) report that the oral administration of magnesium lactate causes a retention of calcium in the presence of an adequate amount of phosphorus.

Evidence in regard to this problem can be seen clearly in the first part of the observations on the two normal medical students (Tables II and III). The addition of the potentially alkaline magnesium lactate, taken by mouth, was accompanied by an increased calcium as well as magnesium excretion in the urine. This is particularly interesting for in our previous studies (20) an alkaline intake has been associated with only an occasional slight urinary increase in calcium output. The stimulating effect of magnesium ingestion on calcium metabolism is even more marked in the subsequent experiment (Periods XXIII to XXVI) when calcium elimination had already been stimulated by the ingestion of ammonium chloride. The addition of magnesium lactate, plus enough added  $\text{NH}_4\text{Cl}$  to maintain approximately the same dietary acidity as in the preceding periods *greatly* increased both the calcium and magnesium in the urine. It is clear, therefore, that the increased calcium excretion following magnesium lactate here, and following magnesium gluconate in F. G. (see Paper II of this series (21)) is due to the effect of the magnesium ion. (The interrelationship of phosphate is discussed later in this paper.)

### *The effect of acid ingestion*

The effect of acid ingestion on calcium metabolism is well known. Phosphoric acid ions have little effect (22) but other anions and acid-producing salts, like ammonium chloride, elevate the calcium elimination distinctly (20). Physiologically, the problem is facilitated by the storehouse of readily available calcium in the trabeculae of bone (23). The calcium, which is excreted because of acid ingestion, we have shown comes largely from these bone trabeculae, but little magnesium is stored there. It is, therefore, of interest to see whether the effects of acids on magnesium and calcium are the same; whether bases can be drawn from soft tissues as well as bone to buffer acid ingestion. From the chemical point of view one might expect magnesium to be so influenced. The effect of these anions on magnesium metabolism has, therefore, been investigated.

Table IV shows the effect of acid ingestion on the magnesium elimination of four subjects. Other studies on these patients have been previously reported, and in order to avoid repetition, references are given in the tables to the papers where additional data may be found.

*Patient A. N.*, aged 16, weighing 34 kilos, had a marked structural scoliosis and was first studied previous to operation. After being on a control neutral low calcium diet for 21 days he was given a diet with a potential acidity of about 700 cc. N/10 per day. After nine days he was given the original control diet to which was added 6 grams  $\text{NH}_4\text{Cl}$  daily. Both the acid diet and the potentially acid salt raised the urinary calcium to a marked degree but the effect upon the magnesium excretion was slight. During his second admission, six months after a spinal fusion, his normal calcium output was greatly reduced, and he was in positive rather than negative balance in respect to magnesium. On the ingestion of  $\text{NH}_4\text{Cl}$  his urinary excretion of calcium was again raised fourfold and there was an increase in urinary magnesium, though not in the total magnesium excretion.

*Patient D. A.*, aged 37, weighing 58 kilos, was studied while recovering from lead poisoning. He was given 8 grams of  $\text{NH}_4\text{Cl}$  daily for six days, followed by two periods with 12 grams per day. As the acid effect of this salt as indicated by

TABLE IV \*  
*Effect of acid ingestion*  
 (Intake and output in 3-day periods)

Subject *	Period	Diet and medication per period	Magnesium				Calcium				
			Excretion			In-take	Excretion			In-take	
			Urine	Feces	Total		Urine	Feces	Total		
A. N. First admission Paper XII (20) Table I	IV V	Control, neutral low calcium	grams	grams	grams	grams	grams	grams	grams	grams	
			.25	.47	.72	.66	.19	.18	.37	.31	
			.27	.49	.76	.66	.16	.16	.32	.32	
	IX X	Potentially acid—ap- proximately 2100 cc. N/10	.33	.38	.71	.52	.71	.14	.85	.30	
			.26	.33	.59	.52	.71	.16	.87	.30	
	XI XII	Control plus 18 grams NH <sub>4</sub> Cl	.47				1.13	.19	1.32	.31	
			.39	.44	.83	.66	1.16	.21	1.37	.31	
	XIII XIV	Control	.26	.26	.52	.66	.66	.21	.87	.31	
			.30	.44	.74	.66	.38	.17	.55	.31	
	SECOND ADMISSION										
		XVIII XIX	Control	.15	.31	.46	.61	.04	.15	.19	.29
				.29	.35	.64	.61	.05	.15	.20	.30
XX XXI		Control plus 18 grams NH <sub>4</sub> Cl	.43	.25	.68	.61	.20	.14	.34	.30	
			.32	.18	.50	.61	.25	.12	.37	.30	
D. A. Paper XII (20) Table II	XIII XIV XV	Control plus 24 to 36 grams NH <sub>4</sub> Cl	.39	.60	.99	.68	.18	.48	.66	.33	
			.49	.75	1.24	.68	.50	.61	1.11	.33	
			.12	.78	.90	.68	.73	.53	1.26	.33	
	XXII XXIII	Control	.21	.34	.55	.68	.07	.48	.55	.35	
.30			.31	.61	.68	.07	.45	.52	.35		
B. E. Paper XII (20) Table IV	III IV V	Control, neutral low calcium	.24	.20	.44	.68	.13	.16	.29	.32	
			.24	.34	.58	.68	.15	.24	.39	.32	
			.25	.34	.59	.68	.18	.25	.43	.32	
	VII VIII IX X	Control plus 12 grams NH <sub>4</sub> Cl	.25	.64	.89	.72	.38	.39	.77	.32	
			.21	.64	.85	.80	.48	.39	.87	.32	
			.21	.52	.73	.80	.46	.22	.68	.32	
			.24	.61	.85	.80	.42	.32	.74	.32	
	XIII	18 grams NH <sub>4</sub> Cl	.29	.68	.97	.80	.65	.33	.98	.32	
	XVI XVII	Control, high calcium plus 18 grams NH <sub>4</sub> Cl	.23	.50	.73	.55	1.02	1.08	2.10	2.11	
			.24	.49	.73	.55	1.03	1.01	2.04	2.11	
	XIX XX	Control, neutral high calcium	.14	.36	.50	.55	.53	.79	1.32	2.11	
			.16	.40	.56	.55	.38	.97	1.35	2.11	
	DeLaB. Paper VI (26) Table III	X XI	Control, high calcium	.15	.25	.40	.55	.009	1.160	1.169	1.570
.15				.40	.55	.55	.008	2.010	2.018	1.563	
XIII XIV XV		Control plus 12 grams NH <sub>4</sub> Cl	.17	.25	.42	.55	.013	1.570	1.583	1.563	
			.18	.35	.53	.55	.022	2.020	2.042	1.553	
			.18	.35	.53	.55	.016	1.660	1.676	1.563	
XVII XVIII		Control plus 18 grams NH <sub>4</sub> Cl	.17	.33	.50	.54	.023	2.010	2.033	1.617	
	.16		.28	.44	.54	.014	1.830	1.844	1.644		

\* See footnote to Table I.

the urinary output of ammonia (24) persisted on into the following periods, these have been omitted in our study on magnesium excretion and two later ones used as control periods. As will be seen from Table IV, the magnesium excretion during  $\text{NH}_4\text{Cl}$  ingestion is greater in both urine and feces than during the two periods on diet alone. The elevation, however, is not so great as that of calcium which was limited to the urinary excretion.

*Patient B. E.*, aged 58, weighing 55 kilos, suffering from chronic sciatic neuritis, was studied while on both a low and a high calcium diet. The addition of  $\text{NH}_4\text{Cl}$  to either diet caused a marked elevation of urinary calcium excretion. The high calcium diet, however, stopped the negative calcium balance, and calcium was actively stored during the control high calcium periods. The magnesium metabolism was also affected in these observations. In spite of a slight increase in the intake of magnesium during the periods of  $\text{NH}_4\text{Cl}$  ingestion, the patient showed a negative magnesium balance in contrast to the control periods when magnesium was stored. In the second observation, made during a high calcium and a lower magnesium intake, the excretion of magnesium is on a distinctly lower level, but still shows the stimulating effect of acid ingestion in spite of the fact that the patient was in calcium balance. The interesting point of this experiment is that when the loss of body fixed base was practically stopped by additions of calcium to the diet, the excretion of magnesium could still be stimulated.

In *DeLaB.*, a patient with steatorrhea and tetany, the ingestion of ammonium chloride resulted in no pronounced elevation of either calcium or magnesium excretion.

The two medical students (M. L., Table II, and R. L., Table III), were also given ammonium chloride. While the urinary calcium excretion of both was increased, the excretion of magnesium was elevated in only one case, R. L., that of M. L. remaining unaffected.

These observations on the effect of the acid-producing salt, ammonium chloride, indicate that magnesium may be used in the body as base, the excess usually appearing in the urine. The amount used is of a smaller magnitude than that of calcium. The amount, however, is too large

to ascribe the source of magnesium to bone alone, and therefore some of it must come from soft tissues. Several of our observations, however, showed that their reaction is not always parallel and that calcium alone could be elevated without influencing magnesium. Likewise, magnesium may be used as base in processes of excretion even if an adequate amount of calcium to produce a positive calcium balance is given in the diet.

#### *The effect of ingestion of phosphate*

In a previous paper (22) it was shown that sodium acid phosphate differed from other acid-producing substances in that it did not increase the excretion of ammonia or calcium in the urine. It was also shown that the ingestion of large amounts of phosphorus either as inorganic salts or as protein had no appreciable effect upon the calcium balance, when the acid effect of the protein was controlled by sodium bicarbonate. Since phosphorus and magnesium are present in fairly large quantities in all body tissues, it would seem not unlikely that the metabolism of one might affect that of the other. It was, therefore, of interest to study the effect of the ingestion and storage of phosphorus upon the metabolism of magnesium. The results of these observations may be seen in Table V.

*Patient W. N.*, aged 34, was suffering from chronic atrophic arthritis. After three control periods, 15 grams of sodium acid phosphate were added to her potentially neutral low calcium diet daily for seven days. No effect on magnesium excretion in urine or feces was obvious.

In *Patient D. A.*, the ingestion of 15 grams of sodium acid phosphate daily for six days resulted in a slightly lower urinary excretion of magnesium than was present in the following control periods though the total excretion of magnesium was practically the same in all periods.

From these two observations, it would seem that the ingestion of acid sodium phosphate has no more effect upon magnesium excretion than upon that of calcium.

*Patient L. Z.*, aged 18, recovering from chronic multiple neuritis, was studied while on a higher calcium intake than most of our other patients. The effect of a high meat diet, the acidity of which was buffered by means of  $\text{NaHCO}_3$ , was first studied. On this diet, urinary calcium was

TABLE V \*  
*Effect of phosphate ingestion*  
 (Intake and output in 3-day periods)

Subject *	Period	Diet and medication per period	Magnesium				Calcium				Phosphorus	
			Excretion			In-take	Excretion			In-take	To-tal excretion	In-take
			Urine	Feces	Total		Urine	Feces	Total			
W. N. Paper XIII (22) Table I	I II III	Control	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
			.12	.31	.43	.59	.24	.39	.63	.29	1.66	1.85
			.19	.29	.48	.59	.33	.46	.79	.28	1.82	1.87
	IV V VI	Control plus 10 to 45 grams $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	.21	.26	.47	.60	.36	.49	.85	.28	2.80	3.85
			.30	.28	.58	.60	.26	.55	.81	.28	8.46	10.84
			.11	.31	.42	.60	.33	.41	.74	.28	8.83	10.84
	VIII IX	Control	.07	.38	.45	.59	.20	.61	.81	.27	1.48	1.85
			.09	.39	.48	.60	.33	.67	1.00	.28	2.02	1.87
	XIX XX	Control plus 12 grams $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	.14	.37	.51	.68	.07	.33	.40	.33	7.58	10.14
D. A. Paper XIII (22) Table III	XXI XXII XXIII	Control	.18	.43	.61	.68	.06	.56	.62	.34	9.73	10.14
			.29	.31	.60	.68	.10	.50	.60	.34	3.88	3.04
			.21	.34	.55	.68	.07	.48	.55	.35	1.91	2.15
L. Z. Paper XIII (22) Table V	I II	Control	.30	.31	.61	.68	.07	.45	.52	.35	1.69	2.15
			.37	.31	.68	.58	1.04	1.23	2.27	1.54	2.41	2.09
	IV V VI VII VIII	High protein plus $\text{NaHCO}_3$	.42	.23	.65	.58	1.01	.67	1.68	1.52	2.44	2.09
			.30	1.08	1.38	1.14	1.20	1.59	2.79	1.55	7.09	6.15
			.28	.93	1.21	1.12	.80	1.38	2.18	1.55	5.84	5.66
			.28	1.05	1.33	1.12	.98	1.40	2.38	1.55	6.38	5.65
			.19	1.31	1.50	.82	.99	1.73	2.72	1.19	5.93	4.83
	X XI	Control	.34	.90	1.24	.98	1.11	1.13	2.24	1.41	6.28	5.29
			.40	.35	.75	.58	1.12	.87	1.99	1.48	3.21	2.05
	XII XIII XIV	Control plus $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	.38	.63	1.01	.58	.91	1.46	2.37	1.55	3.11	2.09
			.31	.26	.57	.58	.86	.56	1.42	1.52	3.95	5.39
			.22	.52	.74	.55	.63	2.15	2.78	1.49	5.24	5.36
	XV XVI	Control	.17	.48	.65	.53	.42	1.09	1.51	1.53	4.69	5.39
			.28			.53	.76	1.00	1.76	1.52	2.10	2.09
			.34	.39	.73	.57	.85	.97	1.82	1.53	1.73	2.09

\* See footnote to Table I.

unaffected, but fecal calcium was increased. The effect on the magnesium excretion is more difficult to evaluate because of the increased ingestion involved in its high concentration in meat. In spite of this, the negative magnesium balance was increased in the feces sufficiently to make a more negative balance than in the control. In Periods XII to XIV the phosphorus intake of this patient was raised to approximately that of the high protein diet by the addition to the control diet of an equimolecular mixture of acid and basic sodium phosphates. During these periods, when phosphorus was being stored, the loss of calcium and magnesium continued as in the control periods

although the excretion shifted slightly from the urinary to the fecal route.

From the above observations, it is clear that magnesium exchange is not greatly affected by the ingestion of large amounts of phosphate and that storage of phosphorus is not necessarily accompanied by retention of magnesium or calcium.

However, a different situation arises when inorganic phosphates are added to a diet already *very high* in magnesium. This can be seen in the observations on the two medical students (M. L. and R. L., Tables II and III). As has already been noted, the addition of magnesium lactate to their adequate phosphate diet definitely

increased the excretion of calcium in their urines. When enough acid phosphate to buffer the large magnesium intake was added to the diet, there was an immediate change from a negative to a positive balance of calcium. This calcium storage was promptly lost after stopping the excess phosphorus and magnesium ingestion. In regard to the *magnesium* balance there is no such consistency, for in R. L. (Table III) the magnesium and calcium are similarly influenced, but the addition of phosphates to the diet of M. L. (Table II) caused no significant alteration in magnesium retention.

It is, therefore, clear that the addition of phosphates to the diet does not increase magnesium or calcium excretion; in fact, phosphate ingestion prevents the stimulation of calcium excretion which is produced by large magnesium ingestion.

#### ✓ SUMMARY

1. Magnesium balance was obtained with hospital patients on an intake of 220 mgm. per day and magnesium storage in active subjects on 300 mgm. per day. These may not be minimal values.

2. There is parallelism of calcium and magnesium metabolism in response to the ingestion of  $\text{NH}_4\text{Cl}$  and both inorganic and organic phosphates.

3. The changes, however, in magnesium metabolism are of much less magnitude than those of calcium, but they are larger than can be accounted for by the amount of magnesium in bone. From this it is apparent that an intracellular kation can be used for neutralizing excess acid ions in urine.

4. Increased magnesium lactate ingestion resulted in an increase in the urinary excretion of calcium, a response which was checked by large intake of sodium acid phosphate.

Magnesium lactate accentuated the effect of ammonium chloride in elevating urinary calcium excretion. It is, therefore, clear that magnesium ingestion increases calcium elimination. ✓

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## MAGNESIUM METABOLISM IN HEALTH AND DISEASE. II. THE EFFECT OF THE PARATHYROID HORMONE

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In this series of studies of the relationship between calcium, magnesium, and phosphorus exchange, the effect of the parathyroid hormone is obviously of primary interest. With the dramatic changes which occur in calcium metabolism, it might be anticipated that similar effects would be found in magnesium exchange, and that this might be evident in soft tissue as well as bone metabolism.

Greenwald and Gross (1) found that thyro-parathyroidectomy in dogs had practically no effect on magnesium excretion. A potent parathyroid extract, however, increased fecal magnesium but had no effect on the urinary excretion (2). After most of the data here recorded had been accumulated, there appeared a publication by Bulger and Gausmann (3), who stated that a loss of calcium is accompanied by a loss of magnesium in hyperparathyroidism and that retention of both accompanies postoperative recovery.

Our data differ somewhat from these observations in untreated hyperparathyroidism. The total magnesium excretion in our three cases of parathyroid adenoma showed no great loss of magnesium. The balances varied; in only one case (G. M.) was there a negative balance, the other two showing a greater storage of magnesium than did our normal controls on a similar intake (4).

However, when comparing the excretion in the same individual before and after parathyroidectomy we find that variations in magnesium do occur, as reported by Bulger and Gausmann (3). These variations are in the same direction though not of the same magnitude as the large changes in calcium excretion.

The observations here reported were all made on patients on the same regime and approximately the same diets as our normal controls described in Paper I of this series (4). The same methods

of analysis were also used. The case histories are appended.<sup>1</sup>

*Patient G. M.* (Table I), a boy of 13, was in a serious toxic state because of an intensely overactive parathyroid adenoma. This condition was entirely relieved by operation. Very careful metabolic observations were made upon him before and promptly after the complete removal of his 11.8 gram parathyroid adenoma. A third observation on exactly the same routine was obtained three months after the operation. These studies were made with both high and low calcium diets in order to observe variations in absorption and excretion which these changes might produce, but no clear cut variation in magnesium metabolism was obvious. The slight changes in excretion were those to be anticipated from the increased magnesium ingestion necessitated by the change in diet.

It is readily seen that immediately after operation there is a marked fall in urinary magnesium excretion and a less dramatic drop in the fecal magnesium. These changes, however, had partly disappeared three months after the operation, though the storage of calcium was then most dramatic. The variations of magnesium metabolism must be interpreted with due regard to changes in weight. Thus, during the pre-operative periods (I to V) and in the observation three months after his operation (XI to XV) his weight was stationary, while he was gaining much weight during the periods immediately after operation. The amount of soft tissue magnesium involved, as well as that which should accompany calcium storage in bone can be roughly calculated. The definite diminution of magnesium excretion in the first postoperative periods is not comparable to the dramatic total change in the calcium balance but is more than ten times as great as the amount to be expected from the calcium balance if the ratio in

<sup>1</sup> These patients are also discussed in another paper (5).



TABLE I  
*Patient G. M., No. 32-599. Parathyroid adenoma*  
 (Intake and output in 3-day periods)

Date	Period	Diet	Body wt.	Vol- ume of urine	Magnesium				Calcium				Phosphorus				Nitrogen		Serum values		Plas- ma phos- phatase
					Excretion			In- take	Excretion			In- take	Excretion			In- take	Excre- tion	In- take	Ca	P	
					Urine	Feces	Total		Urino	Feces	Total		Urine	Feces	Total						
1932			kgm.	cc.	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm. per 100 cc.		units	
May																					
13-20.....	I	Low	32.5	7020	.34	.44	.78	.51	1.95	.48	2.43	.29	2.55	.41	2.96	1.65	21.0	21.8	19.4	4.0	.69
21-23.....	II	calcium	32.5	9020	.31	.46	.77	.51	2.15	.50	2.65	.29	2.52	.46	2.98	1.65	21.5	21.8			
24-26.....	III	High	32.4	9230	.35	.48	.83	.83	2.04	4.03	6.07	4.64	3.00	1.77	4.77	4.60	29.8	33.8			
27-29.....	IV	calcium	32.0	9080	.44	.45	.89	.82	2.27	3.15	5.42	4.63	3.36	1.61	4.97	4.60	33.6	38.1	18.6	4.1	
30-June 1.	V		32.2	8950	.44	.43	.87	.82	2.14	3.06	5.20	4.62	3.44	1.63	5.07	4.60	31.0	36.9			

## PARATHYROIDECTOMY—JUNE 4, 1932

June 12-14.....	VI	High calcium	33.1	4500	.13	.54	.67	.83	.04	3.59	3.63	4.76	1.50	1.77	3.27	4.60	22.0	36.0	8.7		1.70
15-17.....	VII		33.8	4980	.10	.64	.74	.83	.02	3.20	3.22	4.76	1.01	2.18	4.09	4.60	21.3	38.7	8.5		
18-20.....	VIII		34.3	5380	.11	.34	.45	.83	.02	3.12	3.14	4.76	2.21	.93	3.19	4.60	10.8	33.7	7.1	4.0	3.47
21-23.....	IX		34.7	5230	.14	.34	.48	.83	.02	2.32	2.34	4.76	2.40	.98	3.47	4.60	20.3	38.7	7.5	4.0	2.90

## SECOND ADMISSION—SEPTEMBER 8, 1932

September 14-16.....	XI	High calcium	40.7	5300	.36	.42	.78	.83	.02	1.60	1.62	4.76	2.60	.61	3.21	4.60	30.8	38.7	10.4	5.2	1.51
17-19.....	XII		40.7	4980	.35	.44	.79	.83	.02	1.67	1.69	4.76	2.74	.65	3.39	4.60	30.8	38.7	10.4	6.1	
20-22.....	XIII	Low calcium	40.1	3840	.23	.21	.44	.51	.02	.10	.12	.29	1.69	.25	1.94	1.65	19.2	21.8			
23-25.....	XIV		40.6	3400	.22	.20	.42	.51	.01	.11	.12	.29	1.67	.33	2.00	1.65	15.7	21.8			
26-28.....	XV		40.6	4040	.22	.30	.52	.51	.01	.09	.10	.29	1.21	.30	1.51	1.65	15.9	21.8	9.7		
29-31.....	XVI	High calcium		5040	.28	.40	.68	.83	.01	1.42	1.43	4.76	1.93	.70	2.63	4.60	26.4	38.7			
October 1-3.....	XVII	calcium plus viosterol		5440	.32	.36	.68	.83	.04	1.62	1.66	4.76	2.62	.71	3.33	4.60	31.6	38.7	10.9	6.0	.92
4-6.....	XVIII			4940	.30	.33	.63	.83	.02	1.68	1.70	4.76	2.26	.70	2.96	4.60	27.0	38.7	10.5		.83

bones is taken to be  $\text{Ca}:\text{Mg} = 100:1$ . It seems, therefore, justifiable to assume that soft tissue magnesium is involved in the phenomenon. If one assumes a concentration of 20 mgm. per cent of magnesium in soft tissues, the average gain of 400 grams in weight in the first postoperative periods represents 80 mgm. of magnesium which should be stored in each of these four periods. The amount of calcium stored during this time averages 1.68 grams per period, or, at the bone ratio of 100:1, 17 mgm. of magnesium. The sum of these rough approximations—about 100 mgm.—is still less than half the actual average gain in magnesium (245 mgm. per period). Therefore, there was a striking storage of magnesium immediately after correction of this hyperparathyroidism. The observation suggests that much of this reduction is dependent upon soft tissue rather than bone exchange. Three months later, when calcium retention was increased in

intensity, the magnesium excretion still showed a lower level than before operation, though not of great magnitude. This influence is more obvious during the periods of low calcium and magnesium diet, where both urinary and fecal excretion are still reduced. Part of this retention still appears to involve soft tissue magnesium. Here, then, the metabolic exchanges do run parallel, but the calcium retention is of greater intensity and of longer duration.

*Patient A. R.* (Table II), was a woman of 44 years, who had mild symptoms from a parathyroid adenoma, relieved by operation. At her first admission, following x-ray-induced menopause, she showed a nearly normal serum calcium level. During the second observation, six months later, the metabolic evidence definitely indicated parathyroid overactivity. The third study was made six weeks following a successful removal of a small parathyroid adenoma.

TABLE II  
Patient A. R., No. 32-291. Parathyroid adenoma  
(Intake and output in 3-day periods)

Date	Period	Diet	Body wt.	Magnesium				Calcium				Phosphorus				Nitrogen		Serum values (fasting)			Plasma phosphate
				Excretioo			In-take	Excretion			In-take	Excretion			In-take	Excretion	In-take	Mg	Ca	P	
				Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total			
1932			kgm.	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm. per 100 cc.			units
April																					
5-7.....	II	High calcium	83.2	.25	.40	.65	.03	.09	2.38	3.37	5.84	3.90	1.21	5.11	6.43				10.7	1.7	
8-10.....	III		83.2	.27	.45	.72	.03	1.00	2.10	3.10	5.84	4.10	1.17	5.27	6.43						
11-13.....	IV		83.7	.25	.51	.76	.03	.02	1.90	2.82	5.84	4.15	1.08	5.24	5.43				11.2		

## SECOND ADMISSION—OCTOBER 17, 1932

October																					
21-23.....	VI	Low calcium	84.1	.25	.20	.45	.51	.82	.12	.74	.20	1.03	.41	2.34	1.55	20.5	21.8		13.5	2.5	1.36
24-26.....	VII		84.1	.26	.23	.49	.51	.75	.13	.88	.29	1.74	.49	2.23	1.65	20.5	21.8				
30-November 1.....	IX	High calcium	84.4	.32	.29	.61	.93	1.93	.66	2.59	5.84	3.71	.58	4.29	5.44	51.5	58.5				
2-4.....	X		84.2	.38	.44	.82	.93	2.10	1.05	3.16	5.84	3.78	.74	4.52	6.44	49.6	58.5	3.1	13.0	3.4	1.080
5-7.....	XI		83.6	.37	.43	.80	.93	2.08	1.08	3.16	5.84	4.20	.70	4.90	5.44	51.0	58.5	2.8	13.5		.9

PARATHYROIDECTOMY—DECEMBER 2, 1932  
THIRD ADMISSION—JANUARY 18, 1933

1933																					
January																					
26-28.....	XIII	Low calcium	83.9	.15	.36	.51	.51	.02	.30	.32	.29	1.55	.69	2.24	1.65	10.6	21.8		9.8	3.9	.50
29-31.....	XIV		83.4	.21	.22	.43	.51	.02	.19	.21	.29	1.59	.43	2.02	1.55	19.0	21.8		9.9		.55
February																					
5-7.....	XVI	High calcium	83.2	.25	.39	.64	.03	.13	2.08	2.21	5.84	3.44	.91	4.35	5.44	57.4	58.5				
8-10.....	XVII		83.0	.34	.46	.80	.93	.19	2.92	3.11	5.84	3.50	1.20	4.70	6.44	48.7	58.5		0.8	4.2	.56

The data accumulated during the second observation, therefore, represents the period of parathyroid overactivity. The urinary figures indicate a somewhat larger magnesium excretion than in either the first or the third study, just as occurred with the urinary calcium, but the total output of magnesium in the three observations remained the same. In this patient there was a definite storage of magnesium during and after the hyperparathyroid state and the change from a low calcium and phosphorus diet to one very high in both of these constituents had little if any effect on the magnesium metabolism. If the removal of the parathyroid adenoma had any effect upon the magnesium balance, it was not obvious two months after the operation except in regard to the proportion excreted in the urine.

E. M. was a normal male control of 64 years, who had had a severe lead colic three months before the observation. Kept on a constant diet (except for a slight change after the fourth pe-

riod), he received parathyroid extract<sup>2</sup> (Lilly) as indicated in Table III.

The effect on the calcium metabolism was typical and of large proportions. The effect on magnesium exchange certainly was not large, although there was an increase in urinary excretion in Periods IV and V which is more than can be accounted for by the increased calcium excretion from the bones. This elevation was not constant, however, and returned to the control level in the last period, while calcium elimination remained high.

*Can magnesium substitute for calcium in the body?*

Inasmuch as magnesium has apparently often mimicked in small degree the calcium changes which follow acidosis (4) and parathyroid stimu-

<sup>2</sup> We are indebted to Eli Lilly Co. for this parathyroid extract.

TABLE III  
*E. M., No. 33-1159. The effect of parathyroid extract*  
 (Intake and output in 3-day periods)

Date	Period	Diet and medication	Body weight	Vol- ume of urine	Titrat- able acidity plus ammo- nia in urine	Magnesium				Calcium				Phosphorus				Nitrogen		Serum values	
						Excretion			Intake	Excretion			Intake	Excretion			Intake	Excre- tion	Intake	Ca	P
						Urine	Feces	Total		Urine	Feces	Total		Urine	Feces	Total					
1933 September 14-16..... 17-19.....	I	Control, low cal- cium	57.8	7750		.24	.44	.68	.65	.30	.58	.03	.55	.97	2.17	2.14	2.14	26.8	28.6	9.8	3.1
	II		57.5	8520		.26	.35	.61	.65	.30	.51	.08	.43	.79	1.84	2.14	2.14	25.2	28.6	10.0	3.8
	III	Same, plus 1500 units parathyroid extract	56.8	8440	1973	.26	.37	.63	.65	.30	.60	.08	.52	.85	3.11	2.14	2.14	27.7	28.6	11.9	3.8
	IV		57.0	8490		.44	.36	.80	.65	.30	1.32	.84	.48	.73	3.59	2.14	2.14	26.4	28.6	16.4	3.8
26-28..... 29-October 1.	V	Same, plus 750 units parathyroid extract	56.5	7450		.33	.51	.84	.70	.31	1.95	1.34	.61	.74	3.06	2.23	2.23	22.8	28.8	17.1	3.8
	VI		56.7	7870	1194	.26	.34	.60	.70	.31	1.57	1.13	.44	.65	2.53	2.23	2.23	27.2	28.8	15.3	3.2
20-22.....	XIII	Control		6790	1410	.21			.70	.31	.57	.09	.48					26.6	28.8	9.8	3.5

TABLE IV

*Patient F. G., No. 35-69. Parathyroid adenoma. The effect of magnesium ingestion*  
(Intake and output in 3-day periods)

Date	Period	Diet and medication	Body wt.	Volume of urine	Wet weight of stools	Calculated acidity of intake	Magnesium				Calcium				Phosphorus				Serum values (fasting)		
							Excretion			In-take	Excretion			In-take	Excretion			In-take	Mg	Ca	P
							Urine	Feces	Total		Urine	Feces	Total		Urine	Feces	Total				
1935			kgm.	cc.	grams	cc. N/10	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm. per 100 cc.		
January 20-22....	I	Moderate calcium	72.1	6120	215	-10	.27	.30	.57	.91	1.30	1.13	2.49	2.20	2.92	.57	3.49	3.74	2.9	15.5	2.9
23-25....	II			5850	210	-10	.33	.29	.62	.01	1.45	.99	2.44	2.20	3.06	.58	3.64	3.74	2.6	14.2	
26-28....	III	Same plus 17 grams magnesium gluconate		5280	490	-2482	.23	1.25	1.48	3.91	1.24	1.39	2.63	2.20	2.78	.76	3.52	3.74			
29-31....	IV			6390	1030	-2482	.45	3.12	3.57	3.91	1.17	2.10	3.27	2.20	2.67	1.08	3.75	3.74	2.6	14.2	3.0
February 1-3....	V			4710	835	-2482	.44	1.98	2.42	3.91	1.14	1.25	2.40	2.20	2.72	.73	3.45	3.74	2.4	14.1	2.6
4-6....	VI			72.6	6240	985	-2482	.60	2.52	3.12	3.91	1.16	1.72	2.88	2.20	2.67	.88	3.55	3.74	2.5	14.1
PARATHYROIDECTOMY—FEBRUARY 8, 1935																					
February 11....																			2.5	0.5	4.0
16....																			1.4	7.7	
19....																			1.7	8.3	2.7

lation, it is of interest to see whether magnesium can be substituted for the great drain of calcium imposed on the bones by an overactive parathyroid adenoma.

*Patient F. G.*, a woman of 41 years, was a typical case of markedly overactive parathyroid adenoma, with large, multiple bone cysts, a renal stone, and a quiescent duodenal ulcer. In the metabolic research routine she was purposely given a moderately high calcium diet, large enough to produce only a relatively slight calcium loss from the body. After the control periods, magnesium gluconate was added to the regime. If magnesium could substitute for calcium in the body metabolism, then the calcium excretion should be reduced by this addition. The data are collected in Table IV.

The calcium exchange in the control periods, with the very high values for urinary excretion, is characteristic of the disease. The addition of magnesium gluconate to the diet increased the total calcium output in three out of four periods. This increase was entirely due to fecal excretion, as there was a definite decrease in urinary elimination. The average increase in the feces of 560 mgm. of calcium per period might be attributed to the cathartic effect of magnesium gluconate. However, two normal controls studied during three periods of constipation and four periods of

diarrhea induced by cascara showed an average increase in weight of wet feces of 700 grams per period while the increase in fecal calcium excretion averaged but 50 mgm. per period (5). The increase in this case of hyperparathyroidism on magnesium ingestion is, therefore, ten times that found in these controls.

Magnesium was dramatically stored in this whole observation. Even in the control periods it was stored in amounts we have never observed elsewhere.

The phosphorus balance remained essentially unchanged. If the large magnesium retention had been stored as phosphate, a greater retention of phosphorus should have been present in the periods of magnesium ingestion. We are unable to explain this.

From these observations it is obvious that magnesium cannot be substituted for calcium excretion in the hyperparathyroid state. In fact, it increases the loss of calcium (average of 340 mgm. per period). It is possible, however, that the magnesium retention may be substituting for the great loss of calcium from the bones. Evidence for increased relative amounts of magnesium in bones in osteomalacia, rickets, etc., does exist in the literature, though the evidence is by no means unanimous (6, 7). The fact that in this case, with severe decalcification and large

multiple bone cysts, there was a constant large positive magnesium balance; that there was a similar storage even on a very low magnesium intake in a case of steatorrhea with tetany (8); and that in two normal controls on a low calcium intake (4) there was a slight reaction of the same nature, suggests that the calcium lost from the bones may be replaced by magnesium. Direct bone analyses are needed to investigate this.

### CONCLUSIONS

From these observations several conclusions can be stated.

1. The magnesium excretion in the hyperparathyroid state is essentially at a normal level, and two of our three cases stored more magnesium than did the normal controls.

2. Correcting the hyperparathyroid condition is followed, however, by a temporary fall in magnesium excretion, particularly in the urine. This reduction is slight in comparison with total calcium reduction, but is greater than is to be expected from the ratio Mg:Ca in the bones.

3. A few months after parathyroidectomy, the magnesium excretion returns toward the preoperative level, while calcium retention remains intense.

4. The administration of parathyroid extract temporarily raises urinary magnesium excretion to a degree which indicates that some is derived from soft tissue.

5. Magnesium excretion appears to be independent of changes in calcium intake in hyperparathyroidism.

6. The addition of magnesium gluconate to the diet does not relieve the drain upon body calcium due to hyperparathyroidism, as would the administration of calcium. The reverse is true, for increased magnesium ingestion increased calcium excretion.

7. Although magnesium is not substituted for calcium in the excretion during hyperparathyroidism, the storage of magnesium suggests that it may possibly substitute for calcium in the bones.

### CASE HISTORIES

G. M. (C. P. Huntington Hosp. No. 32-599, P. B. B. H. No. 78536), male, was 13 years of age. Never a strong child, he had frequent "bilious" attacks. Three years before entrance he had severe erysipelas. He has

never walked well since, and usually pulls himself upstairs with the aid of his hands. He has always had polydipsia, polyuria, and enuresis, particularly for the last eight months. He complained of pain in his back and increasing weakness and dyspnea. Two months before the present studies he went to the Harvard Dental School where a giant-cell tumor of the jaw was twice removed. The x-ray disclosed a generalized osteoporosis without cysts and without renal calculi.

Physical examination reveals a very emaciated, poorly muscled, tall boy, with moderate beading of costochondral junctions. There is a palpable, soft, walnut-sized mass above the left clavicle over which no bruit could be heard. The left clavicle is disarticulated, muscular development and coordination of movements are very poor. There is a nightly enuresis, unless he is awakened hourly. Laboratory data (not presented in the tables) include:

Basal metabolic rate: + 10 per cent (Aub-DuBois).

Blood: Red blood cells—3,600,000; hemoglobin—65 per cent (Sahli standard 10.1 grams of hemoglobin per 100 cc.).

Urine: Specific gravity never above 1.011. Albumin—trace. Sediment showed few granular casts and a moderate number of pus cells. Phenolsulphonphthalein test 35 per cent. Nonprotein nitrogen 39 mgm. per cent.

After a period of metabolic study the patient was transferred to the Peter Bent Brigham Hospital and on June 4, 1932, Dr. Homans removed a large parathyroid adenoma. This weighed 11.8 grams and histologically was composed predominantly of "Wasserhelle" cells. On June 6 the blood calcium had fallen to 9.0 mgm. per cent. On the sixth postoperative day the patient returned to the Huntington Hospital for continuation of his metabolic studies.

In the four subsequent years the patient has been essentially well.

A. R. (C. P. Huntington Hosp. No. 32-291; M. G. H. series, Case No. 8; path. No. 32-4330), female, was 44 years of age. She felt perfectly well except that for the last five months she had pain in her right hip. At that time (March 1932), x-ray examination showed an extensive destructive process in the right ilium without evident decalcification of other bones. A right kidney stone was visible. At this time, before she had a medical consultation (March 7 to 16, 1932), she was given 1,000 r units of x-ray therapy over her pelvis. This resulted in permanent amenorrhea. On March 16 her serum calcium was 14.8 mgm. per cent, and her serum phosphorus was 1.7 mgm. per cent. On March 18 her calcium was 14.0 mgm. per cent, and her phosphorus was 1.5 mgm. per cent. She was admitted to the Collis P. Huntington Memorial Hospital on March 28, 1932, for metabolic studies. These were repeated in October and November, 1932. On December 2, 1932, she was operated upon at the Massachusetts General Hospital by Dr. Churchill and Dr. Cope, and a parathyroid adenoma of

transition "Wasserhelle" type was removed. Between January 18 and February 17, 1933, she had further metabolic studies at the Huntington Hospital.

Subsequent course: she has been seen repeatedly and has felt perfectly well, though she has gained too much weight. She has had hot flashes and has not menstruated since the x-ray treatment.

F. G. (C. P. Huntington Mem. Hosp. No. 35-69; P. B. B. H. No. 89994) was a married woman of 41 years of age. She had been operated upon for a ruptured gastric ulcer eight years ago and for the removal of a kidney stone four years ago. Since then she has had difficulty in walking because of pain in the legs and lower back. Nine months ago she fell on her back, and x-rays disclosed a generalized severe osteitis fibrosa cystica.

Physical examination showed nothing remarkable except marked pyorrhea, some tenderness over the tips of both tibiae, and abdominal scars.

Extensive laboratory studies showed normal results except that x-ray showed many multilocular cysts, generalized osteoporosis, and a kidney stone. The urine showed consistent low specific gravity. The feces frequently had evidence of occult blood. Serum calcium level was repeatedly above 14 mgm. per cent, and phosphorus level below 2.2 mgm. per cent.

On February 8, 1935, an oxyphil type of parathyroid adenoma was removed by Dr. Zollinger and Dr. Cutler at the Peter Bent Brigham Hospital. This was promptly followed by a drop of serum calcium to 7.0 mgm. per cent, and serum phosphorus rose to 3.7 mgm. per cent. Since then there has been great improvement. X-ray shows the bone cysts are filling in, and except for an increase in weight and a lowered basal metabolic rate the patient finds herself very well.

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# MAGNESIUM METABOLISM IN HEALTH AND DISEASE. III. IN EXOPHTHALMIC GOITER, BASOPHILIC ADENOMA, ADDISON'S DISEASE AND STEATORRHEA

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In an effort to study the relation of magnesium to other inorganic salt metabolism, three diseases other than hyperparathyroidism come to mind. The thyroid gland has been shown to exert an enormous influence on calcium metabolism, and in exophthalmic goiter, even though the blood calcium level is normal, there is the largest calcium excretion found in any disease (1). In steatorrhea is found a very high fecal calcium excretion and often, as a result of deficient calcium absorption, a low blood calcium level with resultant tetany (2, 3, 4). Obviously, it is of interest to study the magnesium excretion in these two diseases.

Addison's disease represents a different problem. It has been shown by Loeb (5) and by Harrop et al. (6) that this disease represents a primary disturbance in sodium chloride metabolism. Whether the calcium or magnesium excretion were abnormal in this disease had not been investigated. A large variation from normal metabolism of either of these bases would not be obvious when total base analyses alone were made, because of their relatively small magnitude in relation to that of sodium. If any close relationship

exists between extracellular sodium and magnesium or calcium metabolism it should be obvious in a study of Addison's disease. Therefore, these three problems have been investigated and are here reported, using the same ward routine and analytical methods as described in Paper I of this series (7).

The magnesium metabolism in exophthalmic goiter is reported in Table I.

The remainder of the metabolic data on these patients can be found in a previous publication (8) and are, therefore, not repeated here. In these two patients with enormously high calcium and phosphorus excretions, the magnesium output is no higher than in our normal controls (7)—in fact, the fecal excretion is somewhat lower and both subjects stored magnesium. Too much stress may not be laid upon the low excretions, however, because these women were small and emaciated, compared to our normal male controls. However, it must be concluded that *exophthalmic goiter does not stimulate magnesium elimination, inasmuch as it is so obviously stored on only a moderately high intake. In this disease, there-*

TABLE I  
*Magnesium metabolism in exophthalmic goiter*  
(Output and intake in three-day periods)

Patient	Period	Basal meta- bolic rate	Wt.	Magnesium				Calcium				Phosphorus				Nitrogen		Serum values	
				Excretion			In- take	Excretion			In- take	Excretion			In- take	Urine	Intake	Ca	P
				Urine	Feces	Total		Urine	Feces	Total		Urine	Feces	Total					
		<i>per cent</i>	<i>kgm.</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	
A. L. (H.H., No. 31-145)...	I	+65	42.8	.39	.22	.61	.81	1.92	1.00	2.92	.33	2.90	.88	3.78	2.20	39.7	41.3		
	11	+65	41.7	.32	.27	.59	.81	2.12	.83	2.95	.33	2.71	.90	3.61	2.25	41.7	47.1	10.4	5.2
R. C. (H.H., No. 30-1485)...	I	+31	57.4	.17	.36	.53	.89	.78	.89	1.67	.32	2.14	.87	3.01	2.34	34.2	47.1	10.4	4.3
	11	+37	56.9	.28	.26	.54	.89	1.33	.67	2.00	.32	2.44	.65	3.09	2.34	43.2	47.1		



TABLE 11  
*Alice D. (H. H. No. 32-451). Basophilic adenoma*

Period	Magnesium				Calcium				Phosphorus				Serum		
	Excretion			Intake	Excretion			Intake	Excretion			Intake	Mg	Ca	P
	Urine	Feces	Total		Urine	Feces	Total		Urine	Feces	Total		mgm. per cent	mgm. per cent	mgm. per cent
1932 I	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams			
April 13-15.....	.16	.20	.36	.55	.65	.27	.92	.30	.87	.47	1.34	1.86		9.8	2.7
II															
April 16-18.....	.12	.41	.53	.55	.84	.48	1.32	.30	.75	1.08	1.83	1.86			
1935 III															
September 20-22.....	.11	.27	.38	.56	.08	.31	.39	.30	1.16	.49	1.65	1.94	2.4	10.0	3.4
IV															
September 23-25.....	.12	.25	.37	.56	.08	.29	.37	.30	1.37	.48	1.85	1.94	2.5	10.2	3.8

fore, magnesium excretion does not parallel that of calcium.

We have also studied an extraordinary case (9) of basophilic adenoma (Cushing's Disease), which is being fully reported elsewhere. The subject (Alice D., Table II) had a remarkable return to a completely normal state following x-ray treatment to the pituitary gland. Metabolic studies before and after treatment disclose dramatic changes in the calcium excretion which are not manifest in the elimination of magnesium. This is another example of the possible independence of excretion of these two kations.

Mrs. DeLaB. (Table III) had steatorrhea with tetany (the history and metabolic findings have been reported in a previous paper (2), as well as by Bauer and Marble (3)). Associated with the low blood calcium there was an extremely low calcium excretion in the urine but a relatively high calcium output in the feces, so that the total calcium output was greater than in our normal controls. In contradistinction to our normal controls, the effect of ammonium chloride ingestion had no effect upon urinary calcium. Ingestion of calcium chloride in large amounts resulted in a large storage of calcium and some elevation of her blood calcium.

The new metabolic data here reported show that the magnesium excretion in the control periods does not appear abnormal. The urinary excretion, while lower than that of some of the male controls, is not different from that of W. N.

(Paper I of this series, Table I) nor of that of the other women in this paper. The fecal excretion is lower than usual, again the reverse of the calcium picture; so that in the control periods there is no parallelism between calcium and magnesium excretion.

In the periods of ammonium chloride ingestion, there is no response either of calcium or of magnesium, so that, obviously, in this patient at least, magnesium could not substitute for a deficient calcium in buffering acid ions in the excreta. Neither did a great shift in the direction of the calcium stream, obtained by giving large amounts of calcium chloride, change the rate of magnesium excretion. It is interesting that on this relatively low magnesium diet, this patient stored magnesium. In the twenty-two periods analyzed during her thirty periods of study, she stored 1.74 grams of magnesium, or 2.6 grams (approximated) for the total time during which she gained 1.7 kgm. in weight. Here, again, is the suggestion that when calcium is lost to the body magnesium is stored.

Therefore, it seems justifiable to summarize this study by the following observations.

1. *Steatorrhea, with its high fecal output of calcium, has a low excretion of magnesium, normally distributed between urine and feces.*

2. *In such a case of calcium deficiency with tetany, magnesium does not replace calcium as a buffer base.*

3. *Calcium may be very actively stored by the*

TABLE III  
*Mrs. DeLaB.—Chronic steatorrhea*  
 (Output and intake in three-day periods)

TABLE III  
Mrs. DeLaB.—Chronic steatorrhea  
(Output and intake in three-day periods)

Diet and medication per period	Period	Magnesium					Calcium				
		Excretion			Intake		Excretion			Intake	Serum
		Urine	Feces	Total			Urine	Feces	Total		
		grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm. per cent
Neutral low calcium diet	III IV V VI	.12 .27 .31 .24	.18 .21 .21 .15	.30 .48 .46	.40 .40 .40 .50	.006 .012 .013 .009	.855 .820 .755 .985	.861 .832 .768 .994	.240 .249 .242 .261	5.1 5.2	
Higher calcium diet	VIII IX X XI	.21 .17 .15 .15	.14 .20 .25 .40	.35 .37 .40 .55	.55 .55 .55 .55	.008 .010 .009 .008	.780 1.090 1.160 2.010	.788 1.100 1.169 2.018	1.448 1.566 1.570 1.563	6.0	
Same diet plus 12 grams $\text{NH}_4\text{Cl}$	XIII XIV XV	.17 .18 .18	.25 .35 .35	.42 .53 .53	.55 .55 .55	.013 .022 .016	1.570 2.020 1.660	1.583 2.042 1.676	1.563 1.553 1.563	6.2	
Same diet plus 18 grams $\text{NH}_4\text{Cl}$	XVII XVIII	.17 .16	.33 .28	.50 .44	.54 .54	.023 .014	2.010 1.830	2.033 1.844	1.617 1.644	7.6	
Same diet—no medication	XX XXI XXII XXIII XXIV	.11 .14 .20 .16 .13	.33 .32 .38 .19 .48	.44 .46 .58 .35 .61	.54 .54 .54 .54 .54	.012 .016 .008 .012	1.650 1.810 2.000 1.000 2.150	1.662 2.016 1.008 2.162	1.644 1.644 1.571 1.694 1.644	7.0 6.8	
Same diet plus 36 grams $\text{NaHCO}_3$ and 4 grams sodium salicylate	XXVI XXVII	.16 .16	.37 .51	.53 .67	.54 .62	.028	1.700 2.100	2.128	1.591 1.595	5.9	
Same diet plus 27 grams $\text{CaCl}_2$	XXIX XXX	.18 .08	.35 .46	.53 .54	.68 .68	.017 .014	3.780 4.700	3.797 4.714	11.374 11.374	7.2	

giving of calcium salts, without influencing magnesium excretion.

4. Magnesium may be stored even over a prolonged period, and on a relatively small amount. The metabolic studies of Table I show that

Her systolic blood pressure was 80 to 68 mm.

giving of calcium salts, without influencing magnesium excretion.

4. Magnesium may be stored even over a prolonged period, and on a relatively small intake.

The metabolic study of Addison's disease (J. F., Table IV, case history appended) was a completely satisfactory observation. The most cooperative subject was in very good health while taking 12 grams of sodium chloride daily and 3 cc. of eschatin (Parke-Davis) twice a week. When these were stopped, she promptly became tired and weak, then bedridden, and on the ninth day without eschatin and the sixth day without salt, because of fatigue and nausea, she had great difficulty in eating all her food in spite of her brave attempts. On the morning of the seventh day of salt starvation, just after the fourth metabolic period was over, she became very weak, perspired profusely, and felt and looked critically ill.

Her systolic blood pressure fell in an hour from 80 to 68. An intravenous injection of normal saline solution plus 3 cc. of eschatin revived her promptly and well. Later, she received 3 cc. of eschatin intramuscularly and sodium chloride by mouth. She promptly recovered her previous strength and, during the last period of the investigation again felt as well as before the study was undertaken.

The results of the investigation indicate that an excessive calcium and magnesium elimination does not accompany the loss of sodium chloride in Addison's disease. Magnesium and phosphate excretion were unaffected, and calcium elimination was diminished in the urine in spite of the large retained with variations in sodium chloride intake in two normal controls (12), which offers a possible explanation for the lowered urinary calcium

TABLE IV  
*J. F. (II. II., No. 35-720). Addison's disease*  
 (Output and intake in three-day periods)

Medication	Period	Volume of urine	Magnesium				Calcium				Phosphorus				Serum values					
			Excretion			Intake	Excretion			Intake	Excretion			Intake	Mg	Ca	Na	K	P	Cl
			Urine	Feces	Total		Urine	Feces	Total		Urine	Feces	Total							
Eschatin + NaCl	I	cc. 4520	grams .25	grams .19	grams .44	grams .45	grams .14	grams .48	grams .62	grams .29	grams 1.01	grams .40	grams 1.41	grams 1.50	m. eq. 1.7	m. eq. 5.1	m. eq. 136	m. eq. 4.2	m. eq. 2.7	m. eq. 112
	II	4940	.20	.18	.38	.45	.14	.49	.63	.29	.95	.42	1.37	1.50	2.0	4.9	135	4.5	2.4	104
No medication	III	6210	.20	.23	.43	.45	.06	.72	.78	.29	1.01	.62	1.63	1.50	2.3	5.2	128	4.2	2.7	98
	IV	5530	.22	.13	.35	.42	.04	.41	.45	.28	1.12	.33	1.45	1.39	2.1	5.5	116	4.4	2.9	94
Eschatin + NaCl	V	3750	.20	.06	.26	.32	.09	.54	.63	.28	1.24	.41	1.65	.95		4.9	128	4.5	2.6	102
	VI	5720	.19	.18	.37	.45	.15	.67	.82	.29	1.26	.49	1.75	1.50	1.7	4.9	132	4.5	2.4	104

in this patient. This diminution in excretion was associated with a significant elevation of the serum calcium. There is a possibility that the elevated serum calcium might be due to an elevated serum protein, which was not determined because of a desire to use a minimal amount of blood. Such an elevated serum protein has been previously described (10).

The article of Rubin and Krick (11) appeared as this article was going to press. They found a reduction of positive calcium and magnesium balances in adrenalectomized rats, which they partially associate with diminished food intake. This relationship in their observations appears to be correct, as our patient who did not suffer from diminished food intake does not show the change in balances which they describe.

This lack of effect on inorganic salts other than sodium chloride is very interesting. With the dramatic fall in serum sodium, and with the large diuresis from body fluids (water intake remained constant) one might well expect an increased excretion of an intracellular base such as magnesium; but the excretion was unaffected and the blood serum level remained within normal limits, and barely changed beyond the limits of error of the determination. The same is true of the blood findings for potassium,<sup>1</sup> the other intracellular

base, but because of this normal blood level we did not study its excretion.

#### SUMMARY

These observations record the study of four diseases which markedly influence the metabolism of several inorganic elements. Exophthalmic goiter has a marked elevation of calcium excretion without change in blood level. A case of Cushing's syndrome also showed a great loss of calcium before treatment, which changed to an excretion below normal after disappearance of the disease. Addison's disease has a high excretion of sodium chloride which is so large it is accompanied by an extensive fall of these ions in the blood serum. However, none of these abnormalities, in which a fundamental change in inorganic salt metabolism is involved, have much effect on magnesium metabolism. In all of these conditions, the magnesium excretion is a little lower than in normal controls, and in pituitary basophilism it remains unchanged in the two observations in spite of the marked changes in calcium excretion. Also, in an individual with steatorrhea, where calcium is absorbed with sufficient difficulty so that a low blood calcium and tetany results, one finds no fundamental abnormality of magnesium excretion.

The extreme specificity of these effects is interesting. One would expect some reciprocal relationship in an organism in which electrolyte levels are so closely guarded. No such influence, how-

<sup>1</sup> The blood analyses of sodium, potassium, and chlorides were very kindly done by Dr. Alan Butler of the Children's Hospital, Boston.

ever, is obvious in these observations. Thus, we have a situation of two kations, closely related chemically, which may behave quite independently in the body. Magnesium, essentially a constituent of cells, may be but little influenced by the factors which affect calcium excretion. Indeed, its rate of excretion is remarkably constant in all the subjects we have studied. Therefore, one must conclude that the usual assumption that calcium, magnesium, and phosphorus necessarily respond as a group is unjustified.

#### CASE HISTORY

J. F. (H. H. No. 35:720) is a 20 year old American telephone operator. For four years she had observed a loss of energy and a gradual loss of nine pounds in weight. Although she had had a fair complexion, for one year she had noticed a progressive increase in universal pigmentation. Four months previous to her entry she had nausea, vomiting, loss of appetite, and such great weakness that she became bedridden. She also had a little diarrhea. She was given 10 grams of salt a day and bi-weekly intramuscular injections of eschatin. A progressive improvement followed this regime so that she felt better than she had for the past year. Physical examination showed a well developed, fairly well nourished girl with no obvious physical abnormalities except a diffuse pigmentation of the entire body which was most marked over the abdomen, the phalangeal joints, and in the creases of the palms. Several patches of brownish pigmentation were present on the gums, as well as in the mucous membrane of the cheeks. Numerous laboratory analyses disclosed normal values. She had a mild secondary anemia of 3,900,000 red cells. The sugar tolerance test showed a slightly elevated blood sugar curve. During medication the blood pressure level varied between 96/45 and 84/40. Her blood plasma volume was 39.5 cc. per kilo—approximately 10 per cent below normal. Biopsy of abdominal skin disclosed hyperpigmentation of skin consistent with Addison's disease. X-ray of adrenals disclosed no calcification.

July 2, 1935—Metabolic routine started.

July 5, 1935—3 cc. eschatin given intramuscularly.

July 6, 1935—First metabolic period started.

July 8, 1935—3 cc. eschatin intramuscularly.

July 12, 1935—Beginning of third metabolic period. Salt intake was lowered from 12 grams to 1 gram daily.

July 15 to 18, 1935—Bedridden, nauseated, very fatigued.

July 17, 1935—Could not eat all the diet.

July 18, 1935—Received 6 cc. eschatin and 20 grams of salt. Fifth metabolic period begun.

July 19, 1935—Back on routine diet with 12 grams of salt.

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# STUDIES IN TEMPERATURE SENSATION. I. A COMPARISON OF THE SENSATION PRODUCED BY INFRA-RED AND VISIBLE RADIATION

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Few of the studies of temperature sensation have emphasized the importance of the perception of thermal change on the regulation of internal body temperature. Not only does the recognition of such changes make it possible for a man to provide a more comfortable environment for himself, but the automatic regulation of heat production and heat loss is dependent primarily upon it. The separate channels through which the human body dissipates its heat have been extensively studied and each of them has been shown to be important under certain environmental conditions. The heat exchange between man and objects in his environment can be altered by increasing or decreasing the rate of blood flow to the skin. The resulting alteration of skin temperature varies the heat exchange by conduction, convection, vaporization, and radiation; and so balances the external heat loss against the heat produced by metabolism that a constant internal temperature is maintained. The recognition by the organism of the temperature of environmental objects is thus important for the regulation of body temperature.

The temperature receptors in the skin which are responsible for the sensation of warmth and cold have been identified (1), and although these end organs are doubtless concerned in the autonomic control of skin temperature, there is no proof that other end organs may not be equally important. However, the study of temperature sensation is the most convenient way of investigating the perception of environmental temperature change.

The knowledge of temperature sensation as it is ordinarily experienced is meagre because almost all experiments have been performed by placing hot or cold objects on the skin so that only the effect of conducted heat or cold has been determined. Such thermal stimulation is always combined with tactile sensation and is quite different

from the stimulation produced by a change of environmental convection or radiation. These latter changes are experienced much more frequently than those due to contact with warm or cold bodies.

There are many reasons why radiant heat is the most suitable stimulus for a study of temperature perception. It is the only thermal stimulus which can be applied without simultaneously provoking another sensation. The magnitude of the stimulus is readily controlled and measured, and the accompanying skin temperature elevation can be determined. The duration of the radiation and the size of the area to which it is applied are unlimited. The use of penetrating radiation has made it possible to produce different types of thermal gradient change during heating and has proved useful in establishing the mechanism by which the end organs in the skin are stimulated by heat. Using radiant heat we have investigated several aspects of temperature perception and have discussed them in the following three parts: Part I considers the absorption of radiation by the skin and the relative effectiveness of radiations of different wavelength on the stimulation of warm sensation. Part II deals with the thermal changes in the skin which are responsible for the sensation of warmth. Part III discusses the minimum sensitivity of the body to temperature change and the effect of the size of the irradiated area on the sensation produced, as it is influenced by the number of end organs stimulated and by spatial summation of the separate end organ responses.

Of all forms of thermal stimuli, radiation is the most frequent and physiologically the most important. Changes in environmental radiation are constantly taking place and a radiation stimulus always precedes a conduction stimulus whenever there is a marked difference of temperature. Furthermore, recent studies (4) have shown that

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the exchange of radiation between the body surface and objects in the environment is the most important mechanism of heat loss under ordinary conditions. Under conditions of comfort about 60 per cent of the heat produced by the body is lost by radiation, and this amount varies considerably under extremes of environmental radiation. In zero weather a man loses twice as much heat by radiation as he produces under basal conditions; and on exposure to the sun on a clear, hot day he absorbs more than three times as many calories as his basal heat production (12). The response to changes of environmental radiation is in itself, therefore, one of the most important mechanisms of heat regulation.

The radiation to which man is exposed in his natural environment comprises a spectral range from  $0.29\ \mu$  in the ultraviolet to  $20\ \mu$  in the infra-red. Physiologically, this range may be divided into four regions, the ultraviolet ( $\lambda < .36\ \mu$ ), the visible ( $0.36\ \mu < \lambda < 0.8\ \mu$ ), the penetrating infra-red ( $0.8\ \mu < \lambda < 3\ \mu$ ), and the non-penetrating infra-red ( $\lambda > 3\ \mu$ ). The infra-red spectrum is so divided because of the intense absorption at the skin surface of radiation longer than  $3\ \mu$ . This absorption is due to the presence of water and organic compounds in the skin (8). The action of ultraviolet light upon the skin is largely photochemical, and this type of radiation does not occur naturally in sufficient amounts to affect the thermal mechanism of the body. The effect of the longer waved radiation, in so far as is known, is entirely thermal. The sun, which is the most important radiator in man's environment, has an energy distribution at the 'earth's surface on a clear day about as follows (11, 13): 5 per cent ultraviolet, 40 per cent visible light, 54 per cent penetrating infra-red, and 1 per cent non-penetrating infra-red. All other bodies in man's surroundings radiate penetrating infra-red or non-penetrating infra-red.

The degree of heating of the skin by radiation depends on the quantity of energy absorbed and therefore is influenced by the reflecting power and penetrability of the skin. These physical properties of the skin have been studied for various spectral regions. Hardy and Muschenheim (9) found that white human skin reflected less than 5 per cent of the non-penetrating infra-red radia-

tion, and that 90 per cent of the energy was absorbed within 0.05 mm. of the skin surface. The reflection of penetrating infra-red was greater, as much as 25 per cent of the short wave near infra-red being reflected and nearly 50 per cent of the remaining energy penetrating to a depth of 0.5 mm. For both regions, the properties of the negro skin for reflection and penetration were found to be the same as those for the white skin. From white skin, more visible than penetrating infra-red radiation was reflected, and visible radiation has been shown to penetrate deeper than penetrating infra-red (2). Negro skin reflects about half as much visible radiation as white skin; the penetration of negro skin has not been satisfactorily measured, but it is probably more opaque than white skin to visible radiation.

A sensory effect of far infra-red, near infra-red, and visible radiation was studied by Sonne (14). He exposed an area on the forearm of white subjects to the maximum amount of radiation from these sources which the subject could bear, and measured the intensity in gm. cal/cm<sup>2</sup>/min. The values were for visible 3.11, for penetrating infra-red 1.79, and for non-penetrating red 1.33. He found that the skin reflected 35 per cent of the visible and penetrating infra-red radiation and none of the longer waved infra-red radiation.

Keller (10) determined the effect of artificial pigment on the heating resulting from irradiation by these sources. He found that painting the skin with india ink had no effect on the heating due to non-penetrating infra-red radiation. However, the same quantity of visible radiation produced a much greater rise in the surface temperature after the pigment was applied than before. The effect of india ink on the heating due to penetrating infra-red radiation was similar to, but less marked than that due to visible radiation.

The present experiments differ from those of Sonne and Keller in several ways. The minimum perceptible sensation has been studied because we believe that it is a more delicate test of warmth perception than the maximum bearable sensation which is essentially painful. Instead of applying pigment to white subjects, negro subjects have been used as a more direct approach to the prob-

lem of pigment function. In addition to the artificial sources of radiation sunlight has been used.

#### METHODS

The sources of artificial radiation which we employed were chosen after careful spectrometric analysis and their emission curves are shown in Figure 1. Non-penetrating infra-red radiation of longer wavelength than  $3\mu$  was obtained from a 250 watt electric hot plate, 12 cm. in diameter. A thousand watt tungsten filament flood light furnished a penetrating infra-red band between  $0.8\mu$  and  $3\mu$  after the visible and long wave infra-red had been filtered out by a Corning heat transmitting glass. This lamp also supplied the visible radiation from  $0.4\mu$  to  $0.7\mu$  after the light had passed through 9 cm. of an 0.8 per cent aqueous solution of copper sulphate. This filter did not completely absorb a small band of infra-red at  $1\mu$  but it furnished the most complete source of pure visible light we were able to obtain. The near infra-red constituted

head of the subject H was held behind a small screen in which a circular aperture,  $14.5\text{ cm.}^2$ , limited an area on the forehead to irradiation. A large screen separated the subject from the operator and allowed the settings for the stimuli to be made without the subject's knowledge. A cardboard shutter was held between the source and a hole in this screen so that rays could pass to the subject only when this shutter was removed. During experiments with visible radiation, special care was taken that the subject did not see the light.

A test for the minimum intensity of radiation which would produce sensation was made in the following way. The subject sat behind the screen with his head in position for some minutes before these experiments began, to accustom himself to the thermal sensation of his forehead. The shutter was then removed and a stimulating amount of radiation allowed to fall on the exposed skin surface for 3 seconds. This length of time was arbitrarily chosen because it was found that if any sensation were produced it would be evoked by irradiation for this

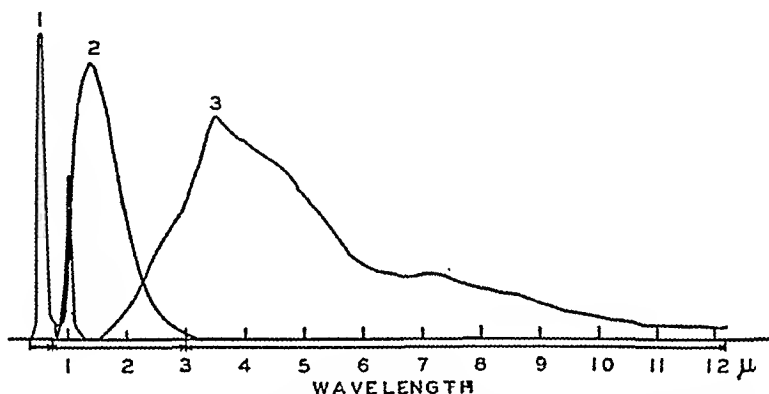


FIG. 1. SPECTRAL DISTRIBUTIONS OF THE ENERGIES OF THE ARTIFICIAL SOURCES OF RADIATION: (1) VISIBLE LIGHT, (2) PENETRATING INFRA-RED, (3) NON-PENETRATING INFRA-RED

about 24 per cent of the transmitted energy, and attempts to decrease the relative amount of near infra-red by altering the thickness or concentration of the filter resulted in absorption of the visible red rays. To obtain a sufficient intensity of visible, and in some cases of penetrating infra-red, the radiation had to be concentrated by a lens. Precautions were taken to keep the focused radiation as uniform as possible over the exposed skin surface. The intensity of various parts of the field was measured, and the maximum variation of intensity was 10 per cent.

The sunlight used for stimulation was directed into the laboratory by a heliostat. The radiation was reflected from two second surface silvered mirrors, and its intensity was regulated by controlling the size of the effective aperture.

A diagram of the apparatus used to measure the stimulating effect of radiation is shown in Figure 2. The source of the radiation, *S*, was mounted on a roll table so that the strength of the stimulus could be altered by moving the source toward or away from the subject. The

time. The subject signalled his perception by calling "on." The intensity of radiation was then decreased by steps, and the test repeated until the smallest intensity of radiation was found to which the subject responded with accuracy. The forehead was then removed, and the radiometer, *R*, placed in the aperture in the screen to measure the strength of the radiation. This strength was designated the minimum stimulus.

False sensations of warmth were largely eliminated by noting the time after the onset of irradiation at which the subject called "on." With radiation of the minimum perceptible strength, the sensation was always noted about 3 seconds after the exposure began. By this means the operator evaluated the perception of sensation reported by the subject. The test was considered satisfactory when the minimum radiation was found to which the subject responded in the characteristic time interval each time he was exposed to radiation. Fatigue and lack of concentration caused a great decrease in the sensitivity of subjects, but when these factors were controlled similar



The ratio of the amounts of these radiations which Sonne found when he produced the maximum bearable sensation in white subjects, is practically the same as that for the barely perceptible warmth. It thus appears likely that this ratio is valid for all degrees of sensation produced by radiation. The sensation which Sonne (14) produced was essentially that of pain and not of heat, a fact which suggests that strong stimulation of the heat receptors results in pain.

negro's pigment influences the absorption of radiation and the sensitivity to radiation. Studies with carbon black are not applicable to an understanding of the functions of the human skin pigment as related to radiation (5, 10).

The relationship between the absorption of radiation by the skin and the sensitivity of both white and negro subjects to it is shown diagrammatically on Figure 3. The basis for the comparison is the sensitivity of white subjects to

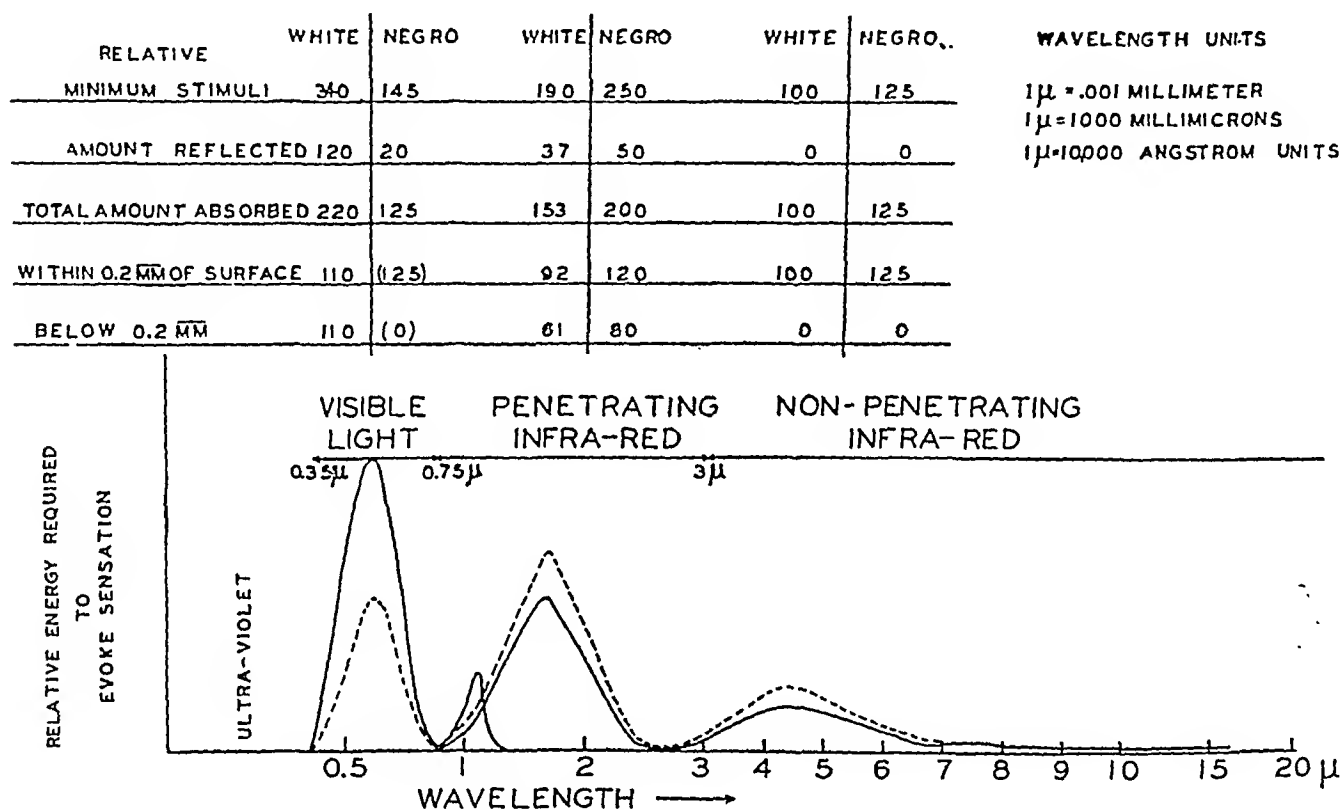


FIG. 3. COMPARATIVE STUDY OF RESPONSE OF WHITE AND NEGRO SUBJECTS TO VARIOUS RADIATION BANDS.  
SOLID LINE: WHITE SUBJECTS. INTERRUPTED LINE: NEGRO SUBJECTS

Minimum stimuli for negroes (Table II), as for white subjects, depend on the reflecting and penetrating properties of the skin. Negro skin is more opaque than white skin to visible radiation and to it the negroes were 55 per cent more sensitive than the white subjects. For both types of infra-red radiation white and negro skin have the same absorbing properties, and the minimum stimuli for these two radiation bands show the same ratio on the two groups of subjects. The fact that the negroes required about 25 per cent more of both infra-red radiations than the white subjects is best accounted for by a difference in the individual sensitivity of the subjects to heat. It is only for the visible spectral region that the

non-penetrating infra-red; it is assumed that 100 units of this radiation are required for stimulation of sensation. For each type of radiation the minimum stimulating quantity has been divided into the amount reflected and the amount absorbed, and the latter has been subdivided into that absorbed at the skin surface (superficial 0.2 mm.) and that which penetrates into the skin. In the lower part of the figure minimum stimuli have been plotted as solid curves for the white subjects and as interrupted curves for the negroes. The only marked difference in these two curves is for visible light and this is associated with a striking difference in the corresponding part of the table. That visible radiation does not penetrate into

negro skin as it does into white skin is shown by this diagram. The absorption by the negroes of only 125 units of visible radiation is required to produce sensation; this is the same as the stimulating amount of non-penetrating infra-red radiation. Since non-penetrating infra-red is completely absorbed within the superficial 0.2 mm. of the skin it is assumed that the visible radiation is similarly absorbed. Because the penetration of visible light into negro skin has not been measured directly, these values on Figure 3 for the energy absorbed at the skin surface and below, are enclosed in parentheses.

In the experiments with sunlight, the white subjects were stimulated by the absorption of about 10 per cent less radiation than the negroes. If allowance is made for the fact that the white subjects were individually about 25 per cent more sensitive to heat than the negroes, the negroes are shown to be about 15 per cent more sensitive to solar radiation than the whites. The 40 per cent visible radiation in sunlight is responsible for this difference in the sensitivity of the two groups. Thus only in sunlight can pigment be considered to play a significant rôle in the exchange of body heat by radiation. It was previously shown that pigment has no effect on the loss of heat from the skin surface (7).

The fact that the least penetrating radiation is the most stimulating, indicates that the production of sensation by radiation results from the activation of the temperature receptors in the skin. It has been suggested by Bazett and McGlone (1) that the sensory response to radiation is due to the stimulation of end organs other than those which usually respond to thermal change. No real evidence has been offered for this theory, and the results of the present study exclude such a possibility. The non-penetrating infra-red which is the most stimulating type of radiation is completely absorbed at the skin surface (within 0.1 mm.) and none of it penetrates to the depths at which the end organs are found. The sensory response to this radiation is necessarily due to heat conducted from the surface.

#### SUMMARY AND CONCLUSIONS

1. Measurement of the thermal sensitivity of the skin to radiation by a new technique is

described. The method provides for an objective determination of the smallest rate of irradiation which will produce sensation. The response of the skin to sunlight, visible light, and infra-red radiation was studied. The infra-red spectrum was studied in two portions, that of wavelength shorter than  $3\mu$  designated as *penetrating* infra-red, and that of longer wavelength designated as *non-penetrating* infra-red. Artificial sources of visible light, penetrating, and non-penetrating infra-red, were obtained by use of proper filters and light sources. The purity of the spectral bands was determined by a spectrometer. The reflecting power of the skin and the intensity of the stimulating energy were measured by a radiometer.

2. The specific stimulating qualities of the various radiations were measured for white and negro subjects. The results with white subjects showed that the minimum amounts of incident radiation required to evoke sensation were in the ratio of 3:2:1 respectively, for the visible, penetrating infra-red, and non-penetrating infra-red radiation. Allowing for the reflected energy the ratio became 2.2:1.5:1. This last difference is attributed entirely to the penetrating power of the radiations; the more penetrating the rays, the less sensitive is the subject to them.

The study of negro subjects gave 1.5:2.5:1.3 as the ratios of the minimum stimulating energies on the same basis as the white subjects. Correcting for reflection 1.3:2.0:1.3 was the ratio. In this study also the difference in the stimulating abilities of the radiation bands can be explained by the difference in the opacity of the skin for the radiation.

3. Comparative studies of white and negro subjects showed that the negro subjects were approximately 76 per cent as sensitive to infra-red radiation as the white subjects. The white subjects were 15 per cent less sensitive than dark negroes to the thermal effects of sunlight.

4. The effect of natural pigment upon the response to thermal radiation is significant only in the visible portion of the spectrum. The effect of natural pigment upon the thermal response to sunlight is found to be small. Therefore, pigment plays only a small rôle in the thermal exchange of man and his environment.

5. The sensory response to radiation is the result of the stimulation of the temperature receptors in the skin.

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# STUDIES IN TEMPERATURE SENSATION. II. THE TEMPERATURE CHANGES RESPONSIBLE FOR THE STIMULATION OF THE HEAT END ORGANS

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In the first paper of this series (10) it was shown that the sensation produced by radiation depends on the absorption of the radiant energy by the skin and is the result of stimulation of the heat end organs. Although many workers have studied temperature sensation there is still no uniform opinion as to what thermal changes activate these end organs. The sensation has been thought to depend on the degree of temperature change (7), the rate of temperature change (12), or on alteration of the normal temperature relationships in the different layers of the skin (4). Bazett (1) and his associates have made the most extensive recent study. They identified the Krause end organs which lie 0.1 mm. deep in the skin, as the warm receptors, and the Ruffini organs at 0.3 mm. as the cold receptors (2), and with small thermocouples placed in the skin attempted to correlate the temperature changes at the end organs with sensation (3). The data did not support any of the common theories of thermal end organ response and could not be explained satisfactorily by any other hypothesis.

An adaptation of the technique that we used in the study of the radiation of heat from the human body provides a new approach to the study of temperature sensation. Without touching the skin, we heated it and measured its surface temperature. The measurements were made by a radiometer designed to determine the skin temperature by measuring the radiation from its surface. This instrument has been found to be more accurate than any others used to measure skin temperature (6). By using radiation to heat the skin, the number of calories applied per second could be readily measured. Furthermore, different types of radiation produce different combinations of deep and surface heating. The penetrating radiation is partly absorbed below the surface so that the deep tem-

perature is elevated at the same time as the surface temperature. Non-penetrating radiation heats only the skin surface. The thermal gradients in the skin could thus be altered by the use of the various types of radiation. While such gradient changes cannot be measured, they can be roughly estimated on the basis of the penetrating properties of the several types of radiant energy. The penetrating properties of visible radiation, penetrating infra-red radiation, and non-penetrating infra-red radiation for human skin were reviewed in the first paper of this series and shown to be quite different. The skin temperature elevations which occurred when minimal sensation is produced by these types of radiation were compared in the present study.

## METHODS

The sources of radiation and the methods of measuring the minimum stimulus and the reflecting power of the skin have been described in Part I of the series (10). The experimental arrangement for measuring the skin temperature is shown in Figure 1, and the parts of the

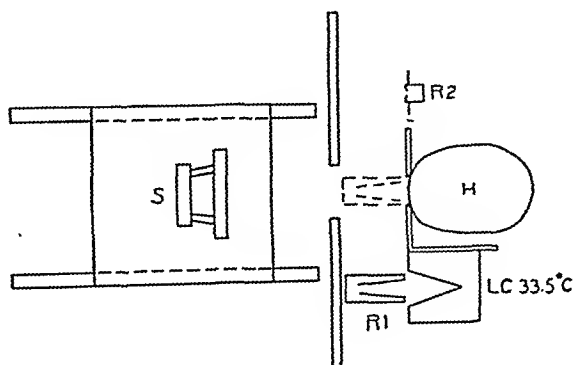


FIG. 1. APPARATUS USED TO MEASURE STIMULATION AND HEATING OF THE SKIN BY RADIATION

S = source of radiation, H = head of subject, R<sub>1</sub> = radiometer for measuring skin surface temperatures, LC = Leslie Cube maintained at 33.5° C., R<sub>2</sub> = radiometer for measuring rate of radiation.

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apparatus used to measure the minimum stimulus are described in the first paper of this series. The radiometer,  $R_1$ , was mounted on a pendulum-like arm so that it could be swung rapidly from its resting position facing the Leslie Cube, to a position facing the skin surface. The construction of this radiometer and its use in measuring skin temperature have been previously described (5). In the present experiments the cone of a Leslie Cube served as a constant temperature reference body, and its temperature was maintained at about  $33.5^\circ\text{C}$ . by a thermostatically controlled heater. Before each skin temperature measurement the temperature of the cube was read, and the difference between the cube and skin temperature measured directly from a galvanometer scale, calibrated so that a difference of  $1^\circ\text{C}$ . caused a deflection of 56 mm.

Skin temperature elevations produced by minimum stimulating amounts of radiation were so small that they were extremely difficult to measure, and such measurements were complicated by the fact that under ordinary environmental conditions the temperature of the skin is always slightly changing. To minimize this difficulty a thermal equilibrium was established between the skin and the air, walls, and other objects in the room before each experiment. When a satisfactory equilibrium had been established, measurements of skin temperature changes could be made with the assurance that whatever changes occurred were due to the experimentally applied radiation. A difference of  $4^\circ\text{C}$ . in room temperature on different days did not appreciably effect the rise of skin temperature caused by radiation. Measurements were made on the forehead because it had been previously found to maintain a more constant temperature than any other part of the body. One other difficulty which interfered with the measurement of skin temperature was encountered, namely, visible and near infra-red radiation were reflected from the skin into the radiometer so that skin temperature could not be measured during irradiation.

To measure the rise of skin temperature due to radiation, the subject sat quietly in a room free from draughts for 10 to 30 minutes before the experiment began and during this interval skin temperature measurements were frequently made. After several successive readings gave the same temperature, the radiometer was returned to the Leslie Cube and the skin surface irradiated for the desired time. Immediately at the end of irradiation, the radiometer was moved back to the skin surface and measurements of skin temperature were made at 5 second intervals, for 60 seconds. From these data the curve for the cooling of the skin after irradiation was plotted. Four curves in Figure 2 show the cooling of the skin after it was irradiated for periods of 15, 30, 45 and 60 seconds. By extrapolating these cooling curves back 8 seconds the skin temperature elevation due to the radiation can be read from the ordinate.

With this method, the results obtained on two subjects for the heating due to radiation were similar and could be repeated consistently. A large number of meas-

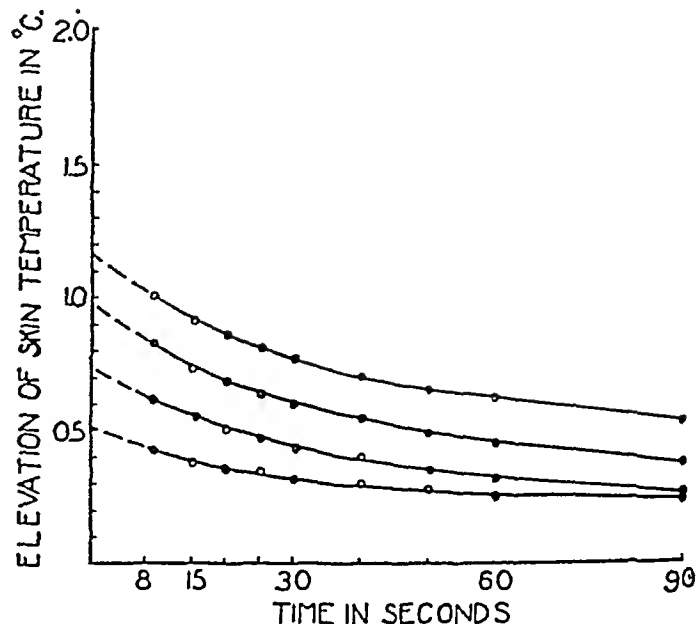


FIG. 2. COOLING OF SKIN SURFACE AFTER RADIATION 15 SECONDS (LOWER CURVE); 20 SECONDS (2ND CURVE); 45 SECONDS (3RD CURVE); 60 SECONDS (UPPER CURVE).

urements with radiation of various intensities were made on these two subjects and the data have been considered together. The values have been recorded in Figure 3 for visible radiation, penetrating infra-red radiation, and non-penetrating infra-red radiation. On this chart the intensity of radiation has been corrected for reflection and is plotted as the abscissa. The skin temperature elevation is recorded as the ordinate. Lines have been drawn between all the points plotted, and these lines indicate the average relationship between the strength of the radiation and the skin temperature elevation produced. The straight lines thus formed show that the temperature elevations are directly proportional to the radiant energy absorbed and demonstrate that the temperature elevations measured are due to the radiation and not to vasomotor or environmental influences.

We have called these charts "constant time charts" and have used them to plot the curves of the heating of the skin produced by any intensity of radiation in the experimental range. Such heating curves are shown in Figures 4, 5 and 6 in which a comparison has been made of the heating of the skin surface by visible, penetrating infra-red, and non-penetrating infra-red radiation.

## RESULTS

In considering the heating of the skin by radiation it is necessary to distinguish between the amount of radiation received by the skin and the amount absorbed by the skin, a difference due to the reflection of visible and penetrating infra-red rays. White skin reflects about 35 per cent of the visible, 20 per cent of the penetrating infra-red, and practically none of the non-penetrating infra-red radiation. Figure 4 shows the heating at the

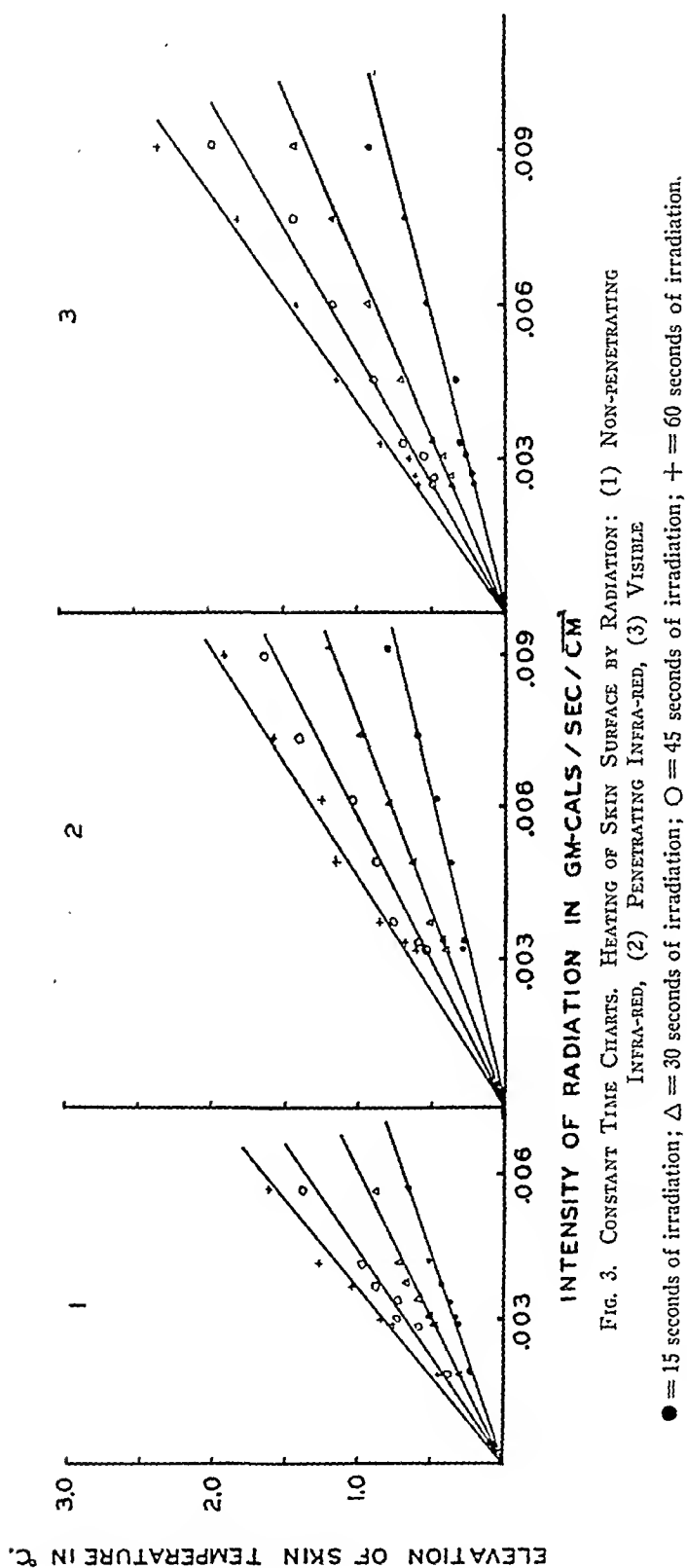


FIG. 3. CONSTANT TIME CHARTS. HEATING OF SKIN SURFACE BY RADIATION: (1) NON-PENETRATING INFRARED, (2) PENETRATING INFRARED, (3) VISIBLE

● = 15 seconds of irradiation; ○ = 30 seconds of irradiation; △ = 45 seconds of irradiation; + = 60 seconds of irradiation.

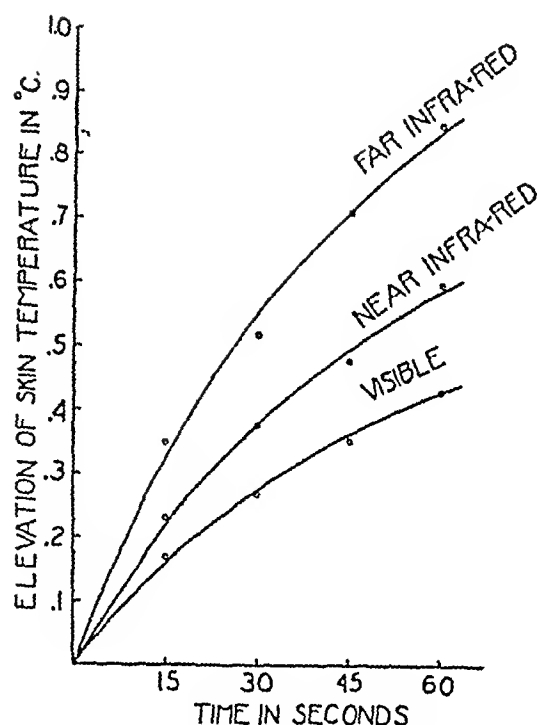


FIG. 4. SKIN SURFACE TEMPERATURE ELEVATION PRODUCED BY THE SAME INCIDENT RATE OF NON-PENETRATING (FAR) INFRA-RED, PENETRATING (NEAR) INFRA-RED, AND VISIBLE RADIATION

surface when the skin receives the same number of gm. cal/cm<sup>2</sup>/sec. of radiation of the three types. The temperature is raised least by the visible radiation and most by the non-penetrating infra-red radiation. When the skin absorbs the same number of gm. cal/cm<sup>2</sup>/sec. of these radiations the difference between the heating curves is, as shown in Figure 5, less marked.

From the constant time charts we determined the number of gm. cal/cm<sup>2</sup>/sec. of the three types of radiation which must be absorbed to produce the same curve for the heating of the skin. The rates of radiation were: visible .0035, penetrating infra-red .0031, and non-penetrating infra-red .0026. When the sources were adjusted so that the skin of the subjects absorbed these amounts of radiation there was a striking difference in the sensation evoked. The non-penetrating infra-red felt much warmer than the penetrating infra-red, and the penetrating infra-red produced a definitely warmer sensation than the visible radiation.

The minimum rates of radiation necessary to produce sensation were determined on the same two white subjects on whom the heating measurements for the constant time charts were made. An area of 7.5 sq. cm. on the forehead was exposed to the radiation. The results of a series of

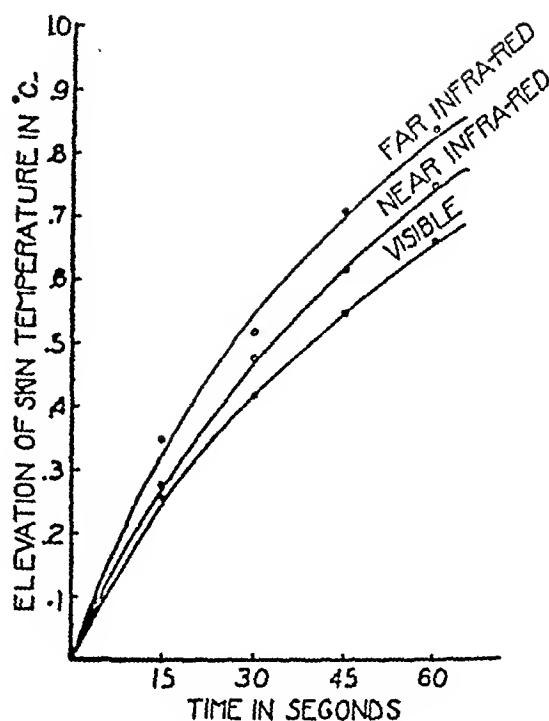


FIG. 5. SKIN SURFACE TEMPERATURE ELEVATIONS PRODUCED BY THE ABSORPTION OF THE SAME RATE OF NON-PENETRATING (FAR) INFRA-RED, PENETRATING (NEAR) INFRA-RED, AND VISIBLE RADIATION

tests are shown in Table I. The average values for the two subjects are: visible radiation .0035 gm. cal/cm<sup>2</sup>/sec., penetrating infra-red .0025 gm. cal/cm<sup>2</sup>/sec., and non-penetrating infra-red .0017

TABLE I  
*Minimum stimuli in gm. cal/cm<sup>2</sup>/sec. of radiation—corrected for reflection*

	Visible		Near infra-red		Far infra-red	
	I	II	I	II	I	II
Subject.....	.0032	.0034	.0023	.0027	.0017	.0019
	.0035	.0036	.0023	.0026	.0016	.0016
	.0036	.0035	.0026	.0025	.0020	.0016
	.0035	.0035	.0026	.0024	.0014	.0016
	.0037	.0036	.0026	.0024	.0017	.0016
Average.....	.0035	.0035	.0025	.0025	.0016	.0017

gm. cal/cm<sup>2</sup>/sec. of radiation. The heating produced by these amounts of radiation is shown in Figure 6. The temperature is raised most by the visible radiation and least by the non-penetrating infra-red. In the following section an hypothesis is suggested to account for the results.

#### COMMENT

There is a difference between the surface and deep temperature changes produced by various

types of radiation because rays of certain wavelength are reflected more or allowed to penetrate deeper than others. Correction for energy loss by reflection shows that there is an important difference in surface heating dependent on the degree of penetration. When all of the energy is

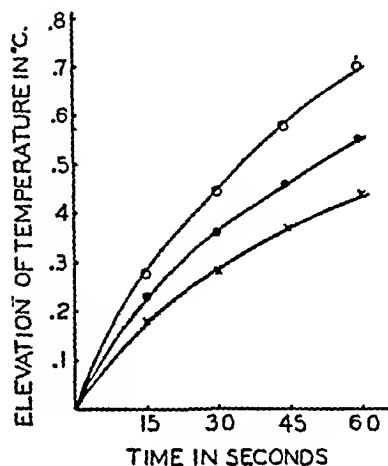
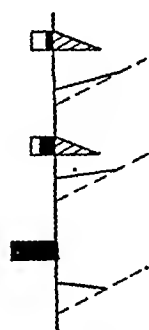


FIG. 6. SKIN SURFACE TEMPERATURE ELEVATIONS PRODUCED BY THE MINIMUM STIMULATING RATES OF VISIBLE (UPPER CURVE), PENETRATING INFRA-RED (MIDDLE CURVE), AND NON-PENETRATING INFRA-RED RADIATION (LOWER CURVE)

absorbed at the skin surface a high surface temperature elevation occurs, and the greater the penetration, the less the surface temperature is elevated. In the deep layers of the skin the temperature is raised higher by the penetrating than by the non-penetrating radiations, a fact illustrated by the experiments of Sonne (11), Keller (8), and Loewy and Dorno (9) who measured the surface and deep temperature during visible, penetrating infra-red, and non-penetrating infra-red radiation. Knowing the penetrability of a radiation it is thus possible to estimate the deep temperature elevation from the surface temperature rise, assuming that there is little or no change in conduction of heat during a short interval of time.

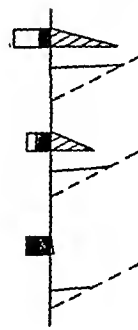
The skin of white subjects reflects 35 per cent of visible radiation and over half of the incident energy penetrates more than 0.2 mm. into the skin. Twenty per cent of the penetrating infra-red radiation is reflected, and less than half of the incident energy penetrates beyond 0.2 mm. Non-penetrating infra-red is not reflected and is completely absorbed at the skin surface. The thermal

#### EQUAL RADIATION



SURFACE DEEP

#### EQUAL SENSATION



SURFACE DEEP

FIG. 7. RELATIVE TEMPERATURE CHANGES PRODUCED IN THE SKIN BY RADIATION

Interrupted lines indicate normal thermal gradient; solid lines, changes produced by the absorption of radiation. Horizontal columns indicate calories of radiant energy. White area is reflected calories; solid area, calories absorbed at skin surface; ruled area, calories absorbed below skin surface.

changes produced in the skin by visible, penetrating infra-red, and non-penetrating infra-red radiation are shown diagrammatically in Figure 7. The strength of the radiant energy is represented by horizontal columns in which the energy reflected is the white part, that absorbed at the skin surface is the solid part, and that which penetrates into the skin is the ruled part. The normal temperature gradient in the skin is indicated by the interrupted lines, and the estimated changes due to the absorption of radiation are shown by the solid lines.

On the left side of this diagram the changes produced by the same incident strength of these three types of radiation are shown. With visible radiation, a small surface temperature elevation is associated with a relatively large deep temperature rise; with penetrating infra-red radiation there is a larger surface temperature elevation and a relatively smaller deep temperature rise; with non-penetrating infra-red radiation the surface temperature elevation is greatest and the deep temperature elevation is relatively smallest.

The right side of this diagram shows the thermal changes produced in the skin by the minimum strength of these three types of radiation which is capable of producing a sensation of warmth. A large amount of visible radiation is needed to



stimulate sensation and high surface and deep temperature elevations are produced. With the penetrating infra-red stimulus the surface and deep temperature elevations are smaller than with the visible stimulus. The non-penetrating infra-red stimulus produces the smallest deep and surface temperature elevations.

The temperature changes produced in the skin by these types of radiation have been considered in detail because a thorough understanding of them is necessary to determine what thermal change is responsible for the stimulation of the heat end organs. Warm sensation depends on one of three possible thermal changes in the skin: the degree of temperature rise, the rate of temperature rise, or a change (or rate of change) of the normal temperature gradient in the skin. The sensation resulting from radiation is due to the warming produced by the absorption of the energy. The same sensation produced by these three types of radiation should therefore be associated with a common thermal change in the skin. It is evident from Figure 7 that minimal sensation resulting from these three types of radiation is associated with a markedly different degree of surface and deep temperature rise. It is also associated with a different rate of temperature rise. The temperature changes shown on Figure 7 are those which occur when the sensation is recognized, that is, after three seconds of radiation. Since the duration of radiation was the same, a difference of actual temperature rise must be accompanied by a difference of rate of temperature rise. Thus, neither the degree of temperature change in the skin nor the rate of temperature change is constant when the same sensation is evoked by these three types of radiation, and neither of these thermal changes can be that which activates the heat end organs.

The only other thermal alteration which could activate the end organs is a change of the normal temperature gradient in the skin. A constant decrease of the temperature gradient in the skin would be shown in Figure 7 by a parallel direction of the solid lines. These three types of radiation are absorbed by the skin in such a way that we would expect them to cause such a constant decrease of the normal thermal gradient with minimal sensation. Our results thus support the hy-

pothesis that a decrease of the normal temperature gradient in the skin is responsible for the sensation of warmth.

This interpretation is open to the criticism that no actual measurements were made of the temperature below the skin surface. We do not believe that it is technically possible to measure the small changes of the thermal gradient which accompany the minimal sensation of warmth. The histological structure of the skin with its vascular and avascular layers does not permit the assumption that the normal thermal gradient is uniformly distributed within the depth of the tissue. Therefore, no calculation of the temperature gradient between two widely separated thermocouples in the tissue is warranted. Local temperature differences can be measured by thermocouples but the only temperature receptors which have been identified lie at the depths of 0.1 mm. and 0.3 mm., and it is not possible to place small wires accurately at these levels. Furthermore, the presence of thermocouples in the skin interferes with the normal temperature relationship. For these reasons we feel that a study of the gradient hypothesis must be indirectly approached and that our experiments have been performed under more natural conditions than those previously reported.

The application to our results of known facts about the heating below the skin surface by these three types of radiation has lead us to support the hypothesis that the stimulation of warm sensation depends on a change in the normal temperature difference between deep and superficial temperature receptors in the skin.

#### SUMMARY AND CONCLUSIONS

The heating of the surface of the skin of white subjects by visible radiation, penetrating infra-red radiation, and non-penetrating infra-red radiation has been measured by a radiometer. It was found that:

1. With the same incident strength of these three types of radiation the skin surface temperature is elevated highest by the non-penetrating infra-red, and least by the visible radiation. Part of this difference is due to the reflection of visible and penetrating infra-red rays from the skin. After correction for reflected radiation there is a smaller difference between the heating of the skin

surface by visible, penetrating infra-red, and non-penetrating infra-red radiation.

2. When minimum perceptible sensation is produced by these three types of radiation the skin surface temperature is elevated higher by the visible stimulus than by the penetrating infra-red stimulus, and higher by the penetrating infra-red stimulus than by the non-penetrating infra-red stimulus.

The application to our results of known facts about the heating below the skin surface by these three types of radiation has led us to conclude that the stimulation of warm sensation depends on a decrease of the normal thermal gradient in the skin and not on the degree or rate of temperature change in the skin.

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stimulate sensation and high surface and deep temperature elevations are produced. With the penetrating infra-red stimulus the surface and deep temperature elevations are smaller than with the visible stimulus. The non-penetrating infra-red stimulus produces the smallest deep and surface temperature elevations.

The temperature changes produced in the skin by these types of radiation have been considered in detail because a thorough understanding of them is necessary to determine what thermal change is responsible for the stimulation of the heat end organs. Warm sensation depends on one of three possible thermal changes in the skin: the degree of temperature rise, the rate of temperature rise, or a change (or rate of change) of the normal temperature gradient in the skin. The sensation resulting from radiation is due to the warming produced by the absorption of the energy. The same sensation produced by these three types of radiation should therefore be associated with a common thermal change in the skin. It is evident from Figure 7 that minimal sensation resulting from these three types of radiation is associated with a markedly different degree of surface and deep temperature rise. It is also associated with a different rate of temperature rise. The temperature changes shown on Figure 7 are those which occur when the sensation is recognized, that is, after three seconds of radiation. Since the duration of radiation was the same, a difference of actual temperature rise must be accompanied by a difference of rate of temperature rise. Thus, neither the degree of temperature change in the skin nor the rate of temperature change is constant when the same sensation is evoked by these three types of radiation, and neither of these thermal changes can be that which activates the heat end organs.

The only other thermal alteration which could activate the end organs is a change of the normal temperature gradient in the skin. A constant decrease of the temperature gradient in the skin would be shown in Figure 7 by a parallel direction of the solid lines. These three types of radiation are absorbed by the skin in such a way that we would expect them to cause such a constant decrease of the normal thermal gradient with minimal sensation. Our results thus support the hy-

pothesis that a decrease of the normal temperature gradient in the skin is responsible for the sensation of warmth.

This interpretation is open to the criticism that no actual measurements were made of the temperature below the skin surface. We do not believe that it is technically possible to measure the small changes of the thermal gradient which accompany the minimal sensation of warmth. The histological structure of the skin with its vascular and avascular layers does not permit the assumption that the normal thermal gradient is uniformly distributed within the depth of the tissue. Therefore, no calculation of the temperature gradient between two widely separated thermocouples in the tissue is warranted. Local temperature differences can be measured by thermocouples but the only temperature receptors which have been identified lie at the depths of 0.1 mm. and 0.3 mm., and it is not possible to place small wires accurately at these levels. Furthermore, the presence of thermocouples in the skin interferes with the normal temperature relationship. For these reasons we feel that a study of the gradient hypothesis must be indirectly approached and that our experiments have been performed under more natural conditions than those previously reported.

The application to our results of known facts about the heating below the skin surface by these three types of radiation has lead us to support the hypothesis that the stimulation of warm sensation depends on a change in the normal temperature difference between deep and superficial temperature receptors in the skin.

#### SUMMARY AND CONCLUSIONS

The heating of the surface of the skin of white subjects by visible radiation, penetrating infra-red radiation, and non-penetrating infra-red radiation has been measured by a radiometer. It was found that:

1. With the same incident strength of these three types of radiation the skin surface temperature is elevated highest by the non-penetrating infra-red, and least by the visible radiation. Part of this difference is due to the reflection of visible and penetrating infra-red rays from the skin. After correction for reflected radiation there is a smaller difference between the heating of the skin

# STUDIES IN TEMPERATURE SENSATION. III. THE SENSITIVITY OF THE BODY TO HEAT AND THE SPATIAL SUMMATION OF THE END ORGAN RESPONSES

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It has long been known that the sensation produced by a heat stimulus of fixed intensity increases with the size of the area stimulated. The observations which have been made on this phenomenon are largely qualitative both as to the magnitude of the stimulus and the size of the stimulated area. Recent investigation of this subject has been made by Bazett and McGlone (3), and Bohnenkamp and Pasquay (4). The former measured the threshold stimulus of sensation for a single heat receptor as a rise in skin temperature of  $0.3^{\circ}$  C. produced at a rate of  $0.2^{\circ}$  C. per second, whereas, the threshold for the whole arm was estimated to be a rise of  $0.1^{\circ}$  C. produced at the rate of  $0.005^{\circ}$  C. per second. Bohnenkamp and Pasquay found a skin temperature rise of  $1.8^{\circ}$  C. as the threshold of a single end organ and a rise of  $0.4^{\circ}$  C. as that for a group of six endings. They discussed their results in terms of spatial summation and expressed their data in an exponential formula of the type of Mach's law of visual intensity discrimination. The nature of these experiments is such that the data are inadequate for a quantitative investigation of the relationship of heat sensation to the size and location of the stimulated area. The purpose of the present experiments is to obtain such data and further, to determine the smallest amount of radiation which can be perceived by the body as warmth.

## EXPERIMENTAL RESULTS

In the present study three variables are considered, namely, the magnitude of the sensation, the strength of the stimulus, and the size and location of the area exposed. The variability of the first of these was eliminated by keeping the sensation constant, i.e., at the minimum value, thereby the relationship between the stimulus strength and the area stimulated could be investigated. The meth-

ods of measuring the minimal stimulus and of obtaining the proper sources of radiation have been described (7). Non-penetrating infra-red radiation was used throughout these experiments because it is invisible, it is more easily obtained in intensities uniform over large surfaces, and it is the radiation for which no corrections for reflection, etc., are necessary. The visible and the penetrating infra-red radiations were used for comparison on the forehead.

A comparison of the sensitivity of forehead areas of different size to visible, penetrating infra-red, and non-penetrating infra-red radiation was first made. Starting with an area of  $0.2 \text{ cm.}^2$ , the area exposed to the radiation was increased by steps using circular apertures of larger diameters. The screens containing the apertures were arranged close to but not touching the skin surface so that normal heat loss from the skin was not affected; the largest circular aperture that could be used on the forehead was one with an area of  $40 \text{ cm.}^2$ . No care was taken to mark the particular skin area tested to insure the testing of the same spot each time, and it was not uncommon for the subject to move his head during an experiment. Thereby many locations on the forehead were tested with the same size area, and the results showed that the sensitivity of the forehead is effectively uniform over its surface. It was not possible to make observations with visible radiation over the whole range of areas because sufficient energy was not available for the small areas and with large areas it was practically impossible to prevent the subject's seeing the light. The sensation results from thermal changes in the skin and these changes are directly proportional to the intensity of the radiation (8). Therefore, in this discussion, the radiation rate is used as a quantitative estimate of stimulus strength. The values given are those for absorbed energy; that is, the incident energy has been corrected for reflection.

<sup>1</sup> New York Hospital Research Fellow.



TABLE II

*Minimum stimuli for various sized areas on the body for non-penetrating infra-red radiation*

Area		Minimum stimulus in gm. cal/cm <sup>2</sup> /sec.			Skin temperature elevation	
Location	Size	Sub-ject I	Sub-ject II	Aver- age	Rate	3 seconds
Forehead	cm. <sup>2</sup>				° C./sec.	° C.
	.20			.0192	0.16	0.48
	.95			.0082	0.060	0.18
	3.46			.0028	0.021	0.063
	7.08			.0017	0.012	0.036
	10.0			.0013	0.009	0.027
	14.5			.00095	0.007	0.0021
	23.8			.0006	0.004	0.013
	40.0			.0004	0.003	0.009
Entire face	197	.00016 .00024	.00024 .00020	.00021	0.001+	0.004
Face and chest	1680 (I) 1940 (II)	.00019 .00016	.00022 .00025 .00019	.00019	0.001+	0.004
Back to waist	1690 (I) 1940 (II)	.00016 .00016	.00022 .00019	.0002	0.001+	0.004
Anterior body surface	5440 (I) 5590 (II)	.00013 .00016 .00013	.00019 .00016	.00016	0.001-	0.003-
Posterior body surface	5440 (I) 5590 (II)	.00013 .00016	.00013 .00016	.00015	0.001-	0.003-
Forearm and hand	360 (I) 420 (II)	.00019 .00019	.00028	.00022	0.002-	0.005

parts exposed which were to be irradiated. For the largest areas the subjects stood nude before the source of radiation. In these experiments a battery of eight hot plates (12 cm. in diameter) mounted vertically 1 cm. apart was used in order to obtain a more uniform distribution of radiation. The radiation varied about 5 per cent over the surface exposed. The results of the tests on two white subjects are given in Table II. The average data for the forehead are included in the table in order to give the entire range of areas studied. The last two columns give the temperature changes occurring at the skin surface during the irradiation. These temperature values are taken from the results of studies reported in Part II of this series (8). The sensation is always perceived after 3 seconds of irradiation and the total change in skin temperature is given in the last column. It is evident that Bazett's value of 0.1° C. as the threshold temperature change for the entire arm is about twenty times too great and the value for the rate of rise about three times too great.

Table II reveals three facts in regard to the area-stimulus relationship. First, after a minimum stimulating energy as small as 0.0002

cal/cm<sup>2</sup>/sec. (or 0.001° C. per second) has been reached, additional area affords no significant increase in sensitivity. This sensitivity is reached when relatively small areas of the body have been exposed such as the head, or the arms, or the chest, etc. Therefore, in ordinary circumstances of life when the body is fully clothed, the radiation sensitivity of the body is as high as it would be if the body were completely exposed. Second, the temperature sensitivity of the body is such that a sensation is evoked by a change in skin temperature of less than 0.003° C., comparing favorably with the most delicate thermometers. The radiation sensitivity is not high compared to a high sensitivity, modern, vacuum thermopile which is sensitive to 10<sup>-12</sup> cal/cm<sup>2</sup>/sec., but compares well with the usual Rubens thermopile which is used in air. Third, as regards the sensitivity the location of an area on the body surface may be more important than its size; the average sensitivity of the face per cm.<sup>2</sup> is more than twice that of the forearm and hand. This is an expected result as it is well known that certain parts of the body are more sensitive to heat stimuli than others.

The back and arm were tested and it was found that the areas were highly variable in sensitivity from place to place, and nothing could be made of the data without further knowledge of end organ distribution. The hand and forearm were care-

TABLE III

*Minimum stimuli for various localities on forearm and back of hand with an exposed area of 23.8 cm<sup>2</sup>. Far infra-red stimulation*

Area	Subject I	Subject II	Average
Back of fingers	.00075 .00084 .00110	.00084 .00110 .00081	.00091
Back of hands (metacarples)	.00088 .0010 .0011	.00075 .00085 .00088	.00091
Back of wrist	.0019 .0015 .0019	.0019 .0019 .0017	.0018
Back of forearm (10 cm. from wrist)	.0022 .0020 .0021	.0024 .0025 .0027	.0023
Back of forearm (5 cm. from elbow)	.0041 .0039 .0036 .0035	.0045 .0016 .0042 .0035	.0040

TABLE I

*Minimum stimuli in gm. cal/cm<sup>2</sup>/sec. for various sized areas of the forehead for non-penetrating infra-red, penetrating infra-red, and visible light*

Area	Non-penetrating infra-red			Penetrating infra-red			Visible		
	Sub-ject I	Sub-ject II	Aver-age	Sub-ject I	Sub-ject II	Aver-age	Sub-ject I	Sub-ject II	Aver-age
cm. <sup>2</sup>									
0.20	.0205 .0186	.0189	.0192						
0.95	.0084 .0087	.0092 .0065	.0082	.0157 .0162	.0158	.0159			
3.46	.0028 .0027 .0030	.0028 .0030	.0028	.0059 .0055	.0055 .0050 .0065	.0057			
7.08	.0018 .0019	.0018 .0014 .0015	.0017	.0027 .0028	.0026	.0027	.0035 .0031 .0036 .0035 .0035 .0036	.0034 .0036 .0035 .0035 .0035	.0035
10.0	.0014 .0013	.0011 .0013	.0013	.0020	.0020 .0020	.0020			
14.5	.0008	.0010 .0009 .0009	.00095	.0012	.0012 .0012	.0012	.0016 .0016 .0017 .0018 .0017	.0017 .0017	.0017
23.8	.0005	.0006 .0006	.0006	.0008 .0007 .0007	.0011 .0010	.0009			
40.0	.0004	.0004 .0005	.0004	.0007 .0008	.0008 .0007	.0007			

The results of tests on two white subjects with the three types of radiation stimuli are tabulated in Table I. Each value represents a complete test which usually included several individual trials. Column 1 of the table contains the size of the areas tested, Columns 2, 3 and 4 the corresponding amounts of radiation in gm. cal/cm<sup>2</sup>/sec. which were required to produce a barely perceptible sensation of warmth. It will be noted that the per cent of variation in the individual determinations is about the same for all sizes of areas. The decrease in the amount of stimulus required to evoke sensation as the area irradiated is increased, confirms in a quantitative way the qualitative observations of previous workers.

As pointed out in an earlier paper (7), the difference in the stimulating power of these radiations depends on the manner in which the skin absorbs them: that is, the more penetrating the radiation the less effective it is in stimulating sensation. This difference is seen to exist for all areas on which comparisons were made, and the ratio of the stimulating energies is approximately

the same for all the areas. It follows, therefore, that the number of end organs stimulated in any area is the same for all three types of radiation, and the penetrating radiations do not stimulate end organs which might be located at greater depths in the tissues any better than the non-penetrating radiation. Histological evidence (1) indicates that temperature end organs may be distributed in the tissues at depths from 0.1 mm. to 1 mm. but they respond to heat stimuli as if depth were of no consequence. Anatomical evidence (1, 4) also points to a rather sparse distribution of end organs over the skin surface (i.e., one ending per sq. cm. on the prepuce and forearm). End organ counts on the forehead were not made, but the evidence from heat measurements is that the sensitivity of the forehead, even for small areas, is nearly uniform from place to place. This would indicate either that the forehead is densely populated with receptors or that each end organ responds to stimulation over a considerable skin area. This latter possibility as well as the fact that penetration of radiation does not increase the number of end organs responding, supports the idea that the endings respond to the thermal changes occurring in a blood vessel network which forms a layer of high thermal conductivity under the skin surface. The venous and arterial plexuses of Spaltcholtz probably represent such a layer.

The close analogy between such a system and the physicist's radiation thermopile is interesting. The usual type of radiation thermocouple has soldered to it a metal disc or radiation "receiver" which has considerably greater area than the thermocouple itself. In the same way, although the nerve ending be punctiform, the circulation of blood in its neighborhood forms a highly conducting "receiver" for heat stimuli.

Tests were made not only of the forehead area but of the whole of the face, the thorax, and the anterior body surface with non-penetrating infra-red radiation. The size of the exposed areas was measured by taking the silhouette upon paper and determining the area either with a planimeter or by weighing. The back of the thorax and the entire posterior body surface were also tested. Except for the experiments on the whole body surface, the subjects were seated with only those

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Face and chest	1680 (I) 1940 (II)	.00019 .00016	.00022 .00025 .00019	.00019	0.001+	0.004
Back to waist	1690 (I) 1940 (II)	.00016 .00016	.00022 .00019	.0002	0.001+	0.004
Anterior body surface	5440 (I) 5590 (II)	.00013 .00016 .00013	.00019 .00016	.00016	0.001-	0.003-
Posterior body surface	5440 (I) 5590 (II)	.00013 .00016	.00013 .00016	.00015	0.001-	0.003-
Forearm and hand	360 (I) 420 (II)	.00019 .00019	.00028	.00022	0.002-	0.005

parts exposed which were to be irradiated. For the largest areas the subjects stood nude before the source of radiation. In these experiments a battery of eight hot plates (12 cm. in diameter) mounted vertically 1 cm. apart was used in order to obtain a more uniform distribution of radiation. The radiation varied about 5 per cent over the surface exposed. The results of the tests on two white subjects are given in Table II. The average data for the forehead are included in the table in order to give the entire range of areas studied. The last two columns give the temperature changes occurring at the skin surface during the irradiation. These temperature values are taken from the results of studies reported in Part II of this series (8). The sensation is always perceived after 3 seconds of irradiation and the total change in skin temperature is given in the last column. It is evident that Bazett's value of 0.1° C. as the threshold temperature change for the entire arm is about twenty times too great and the value for the rate of rise about three times too great.

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The back and arm were tested and it was found that the areas were highly variable in sensitivity from place to place, and nothing could be made of the data without further knowledge of end organ distribution. The hand and forearm were care-

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Back of hands (metacarples)	.00088 .0010 .0011	.00075 .00085 .00088	.00091
Back of wrist	.0019 .0015 .0019	.0019 .0019 .0017	.0018
Back of forearm (10 cm. from wrist)	.0022 .0020 .0021	.0024 .0025 .0027	.0023
Back of forearm (5 cm. from elbow)	.0041 .0039 .0036 .0035	.0045 .0046 .0042 .0035	.0040



fully studied and will serve to illustrate the situation. The sensitivity of the various localities on the forearm and hand was measured using one size of aperture and non-penetrating infra-red stimulation. The results are shown in Table III. The hand was found to be more than four times as sensitive as the proximal part of the forearm. The forearm was found to be progressively less sensitive nearer the elbow, suggesting a decreasing end organ population. Recognizing the presence of this variable, which was not significant when dealing with the forehead, tests were made by successively increasing the size of the area exposed. By means of a movable shutter the arm was gradually exposed by known amounts from the fingertips to the elbow and from the elbow to the fingertips. The results are shown in Table IV. Although the forearm and hand are

TABLE IV

*Summation stimuli for forearm and back of hand*

Direction of area increase: Fingertips to elbow  
Distance from fingertip

cm.	Area cm. <sup>2</sup>	Stimulation
4.0 (Hand).....	20	.0013
7.0 (Hand).....	40	.00065
12.0 (Hand).....	100	.00053
20.0 (Forearm).....	170	.00041
30.0.....	240	.00029
47.0.....	360	.00019

Direction of area increase: Elbow to fingertips  
Distance from elbow

6.5 (Forearm).....	65	.0015
9.5 (Forearm).....	100	.0011
14.0 (Forearm).....	130	.00069
22.5 (Forearm).....	190	.00044
47.0 (Hand).....	360	.00019

not per unit of area as sensitive as the forehead, they combine to form an area which is nearly as sensitive as the whole body surface. Thus a paucity of end organs is made up for in large degree by the efficient combining of the separate effects of each end organ.

In order to test further the combining power or spatial summation of various portions of the body the following experiments were performed. Two square apertures (25 cm.<sup>2</sup> area, 3 cm. apart) were so arranged that the skin areas placed behind them could be tested singly and together. The procedure was to test each aperture alone and then together. It was found that on the forehead the contiguity of the area did not affect the values of

the stimuli. Thus, the right and left apertures gave identical values when stimulated separately; together they gave a value to be expected from the curve shown in Figure 1. Tests were then made on the backs of both hands, testing each hand separately and then both together. The same tests were made using as combination, the right side of the forehead and the back of the right hand. The results are shown in Table V.

TABLE V

*Summation stimuli for various body areas*

Subject I				Subject II			
Left	Right	Both	Summa- tion	Left	Right	Both	Summa- tion
BACKS OF HANDS							
.0009	.0009	.0006	per cent	.0012	.0013	.0008	per cent
.0008	.0008	.0006	47				48
		.0006					
		.0006					
		.0006					
BACK OF RIGHT HAND AND FOREHEAD							
Fore- head	Hand	Both	Summa- tion	Fore- head	Hand	Both	Summa- tion
.0005	.0011	.0005	0	.0006	.0012	.0006	0

The sensitivity of both hands together was 47 per cent greater than either hand alone, although simultaneous stimulation of the hand and the forehead showed no increase in sensitivity over the forehead stimulated alone. This is evidence that spatial summation of heat sensation may have a value of zero and that the magnitude of the sensory effect is greatly dependent upon this factor. The close association of the hands suggests that habit may be important in the degree of sum-

TABLE VI

*Summation values for forehead*

Area	Stimulus	A × I	Antilog (log I + .78 log A)	Relative summation
cm. <sup>2</sup>	cal/cm <sup>2</sup> /sec.			per cent
0.95	.0082	.0078	.0081	
3.46	.0029	.0097	.0078	75
7.08	.0017	.0120	.0079	66
10.0	.0013	.0130	.0078	59
14.5	.0009	.0138	.0078	55
23.8	.0006	.0143	.0071	61
40.0	.0004	.0160	.0079	44

mation which is attained between different parts of the body.

### DISCUSSION

1. *Subjective phenomena.* A description of the impression reported by the subjects during stimulation of minimal sensation on the various exposed areas, is of assistance in interpreting the data. Stimulation of the small areas, less than 7 cm.<sup>2</sup>, required so intense a rate of radiation that pain would result upon exposure of a large area to it. The minimal sensation for these small areas was more of a "hot" than a "warm" sensation. For the areas between 10 cm.<sup>2</sup> and 200 cm.<sup>2</sup> the sensation was that of a fleeting warmth lasting only a few seconds. At times the characteristic of "warmth" seemed to be lost, and the subject felt he was reporting a "change" of conditions rather than a feeling of warmth. For larger areas, the intensity of the sensation increased as the area is increased, and a definite feeling of warmth was perceived. When the minimum stimulus for the face was applied to the whole body surface the subject perceived a marked sensation of warmth, and it was always with some surprise that he found that he could not perceive a smaller rate of radiation. The area for which an intensity of 0.0002 cal/cm<sup>2</sup>/sec. is the stimulus is in this sense a critical area, because for larger areas sensation is no longer minimal, and the minimal stimulus is constant.

2. *Graphic analysis.* Analysis of the data of Table II can be made by a graphical representation. A graph of the values in numerical coordinates is a curve of the type of an equilateral hyperbola and such a function is more easily handled in logarithmic coordinates. A plot of the data in terms of the logarithms of the area and stimulus is shown in Figure 1. The whole curve is definitely sigmoid although the portion from 1 to 40 cm.<sup>2</sup> on the forehead is a straight line. This form of curve is familiar to those studying visual sensation (6) and like the curve for visual acuity can be most easily studied in three parts: (I) the portion for very small areas (less than 1 cm.<sup>2</sup>), (II) the portion which is a straight line, and (III) the portion for very large areas.

3. *Lower portion of log curve (excitation threshold).* The breaking of the log curve at the stimulus 0.0002 cal/cm<sup>2</sup>/sec. is strongly suggestive of the failure of some physiological process. The increase in sensation for large areas without increase in sensitivity shows that the summation process does not fail at this stimulus value. As a consequence, 0.00016 cal/cm<sup>2</sup>/sec. must represent the *excitation threshold* of the most sensitive heat receptors. Thus any greater intensity of stimulus will cause the end organs to respond and a stimulus smaller than this will evoke no effect regardless of area.

The region of Figure 1 may, on the basis of

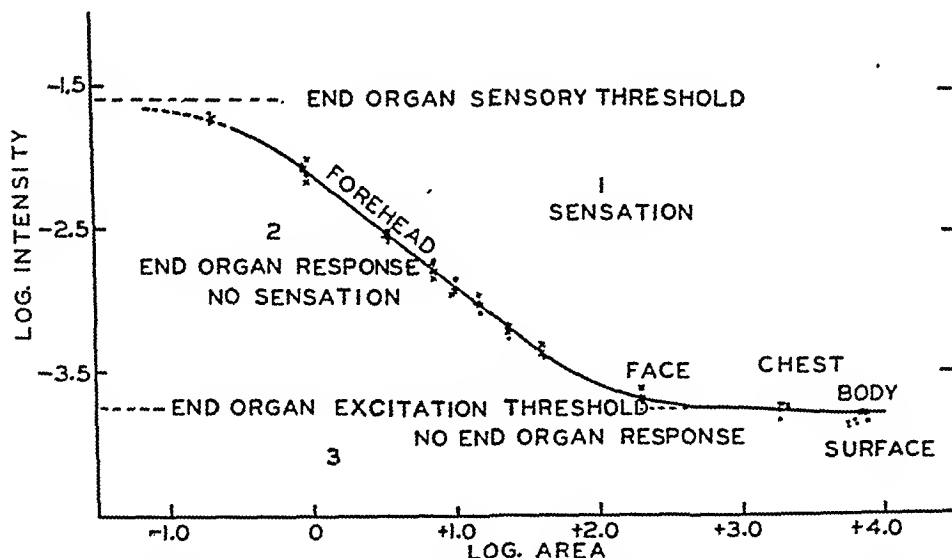


FIG. 1. RELATIONSHIP OF THE LOGARITHMS OF STIMULUS INTENSITY AND AREA STIMULATED

the above interpretation, be divided into three parts each having a physiologically different meaning. Area I, above the log curve, represents stimuli magnitude which will evoke sensation; areas II and III below the line refer to subthreshold sensory stimuli. In area III, below  $0.00016 \text{ cal/cm}^2/\text{sec.}$  ( $\log I = -3.79$ ), no end organ response is evoked, and in region II, above  $0.00016 \text{ cal/cm}^2/\text{sec.}$ , end organs are responding but no sensory impression is made. The physiological effect of these unfelt impulses is not known at present.

4. *Upper portion of log curve (sensory threshold).* The breaking of the log curve for small areas and strong stimuli is an expected result. When an intensity of radiation has been reached which will evoke a sensation from one end organ, further decrease in area will require no increase in stimulus as long as the end organ remains in the irradiated field. Therefore, the intensity of stimulus for which the log curve again becomes parallel to the area axis is the threshold of sensation for a single receptor. This value cannot be accurately determined from the present data because very small areas were not tested. However, the value evidently lies between  $0.031$  and  $0.025 \text{ cal/cm}^2/\text{sec.}$ , corresponding to a temperature change of  $0.16^\circ \text{ C.}$  per second with a rise of  $0.48^\circ \text{ C.}$  in three seconds. These temperature values are in almost exact agreement with the measurements made with temperators on the prepuce by Bazett and McGlone (2). The area at which the break in the curve starts is  $0.2 \text{ cm}^2$  so that one may roughly estimate that one end organ is present in an area of about this size. The number of endings may be considerably larger if one takes into account the presence of receptors of higher threshold, and the above estimate would represent the minimum population.

5. *The central portion of the log curve.* The two parts of the log curve which have been discussed are those for which change in size of area stimulated caused no change in sensitivity. The straight line relationship between log intensity and log area, which is seen to be valid for the forehead is significant only when the special character of the forehead is considered. Over the forehead area the sensitivity of the skin per  $\text{cm}^2$  is uniform, a condition we did not find on other parts

of the body surface. On the basis that uniformity of sensitivity within this area means a nearly uniform distribution of heat end organs per  $\text{cm}^2$ , the data for this area can be partly analyzed. The data may be represented by the formula

$$1. \log I + .78 \log A = -2.09,$$

where  $I$  = intensity of radiation,  $A$  = area irradiated.

Thus, as the area stimulated is doubled the intensity is almost halved. The fact that the intensity does not decrease in *exactly* the same proportion as the number of available endings is increased is analogous to the multiple junction thermocouple. Due to increased resistance, the sensitivity of a thermopile does not double if the number of junctions is doubled. This falling off of sensitivity may be due to the progressive failure of the summation of the effects of the separate end organs or to a smaller number of end organs per unit area responding to the lowered intensity (6). The present experiments do not throw light on this matter.

Equation 1 is not the type of formula suggested by Bohnenkamp and Pasquay (4) and the present data cannot be fitted to their equation. However, their observations covered only six endings and are not directly comparable with these observations.

It is interesting to note the formal identity of Equation 1 with the formula for area and intensity found by Granit and Harper (5) when investigating spatial summation in the retina. Their equation is approximately

$$2. \log I + c \log A = \text{constant.}$$

6. *Spatial summation.* The ability to add the separate effects of end organs in widely distributed areas varies from zero, in case of the forehead and hand, to 47 per cent for the backs of the right and left hands. The summation within the forehead area is almost perfect. Impulses from different areas of the chest are added but the summation has not been quantitatively studied. Areas of poor sensitivity sum with areas of high sensitivity as has been shown for the forearm and hand. It would seem that summation depends to some extent upon habit and association, but the matter has not been completely investigated.

That spatial summation is of prime importance in determining the intensity of heat sensation is clearly demonstrated. So far as our experiments go, no summation as high as 100 per cent has been observed and no clear examples of facilitation nor occlusion have been found.

7. *Intensity discrimination.* Intensity discrimination for heat sense has not been measured although it can be seen from the present experiments to be poor. The total range of intensities from end organ excitation threshold to pain is only 200-fold. The magnitude of the variations ( $\Delta I$ ) in the measurements of the minimum stimuli over the range of intensities is proportional to the intensity ( $I$ ). This is the type of relation which would be expected on the basis of a "Weber-Fechner" law for heat sensation, a relationship which might be suspected from the similarity of the formula for vision and heat sense.

8. *Sensitivity of body to heat.* The temperature sensitivity of the body surface shown in Table II is evidence of the remarkable heat detector mechanism by which the internal temperature of the body is regulated. At the minimum perceptible rate of radiation the sensation is evoked usually after 3 seconds and the total rise in skin temperature under these circumstances is less than  $0.003^{\circ}\text{C}$ . Such high sensitivity might be expected from the important part which the skin temperature plays in the normal loss of heat from the body surface. About 75 per cent of the heat produced in the body under comfortable circumstances depends upon the difference between the skin temperature and the surrounding temperature. A change of  $1^{\circ}\text{C}$ . in the temperature of the walls alone, or 10 per cent in the normal radiation rate, air temperature, velocity, and humidity, etc., being constant, will evoke a subjective sensation in three seconds. The energy exchange in this process is of the order of one nine millionth of the normal hourly heat loss by radiation. That a sensation need not be necessary for this regulatory process is pointed out above, as the evidence shows that end organ responses may be going to the central nervous system, without evoking a sensation. These impulses may have connections with vasomotor pathways to the skin so that adjustment of internal thermal gradients can

be made with a consequent decrease in end organ activity. In this way the surface temperature of the body could be adjusted to changing conditions without the sensory response reaching the level of awareness.

#### SUMMARY AND CONCLUSIONS

1. The sensory response produced by irradiation of a large range of areas of the body surface has been studied. The method allows stimulation of skin areas as large as the surface of the experimental subject and as small as desired. Tests were made of the minimum stimulating radiation rate for many parts of the body and for the body as a whole. Measurements were also made with radiations of different wavelengths.

2. For the whole range of intensities covered in this study the non-penetrating radiation was found to be more effective in stimulating sensation than the penetrating forms. The variation in depth of the end organs below the skin surface must therefore be unimportant as a factor in the thermal sensitivity.

3. The smallest rate of radiation which the body is capable of perceiving as warmth is  $0.00015\text{ gm. cal/cm}^2\text{/sec}$ . Sensation is evoked in 3 seconds by exposure of  $200\text{ cm}^2$  of surface to such a stimulus so that the total energy exchange for sensation is  $0.09\text{ gram cal}$ . This amounts to one nine millionth of the normal hourly radiation loss from the body surface.

4. The skin temperature change caused by this radiation is a total elevation of  $0.003^{\circ}\text{C}$ . produced at the rate of  $0.001^{\circ}\text{C}$ . per second.

5. The *intensity* of the stimulus (rate of radiation) necessary to evoke sensation increases as the area stimulated is decreased, although in all cases the *magnitude* of the stimulus (total heat received) decreases as the area exposed decreases.

6. The forehead was found to be an area over which the sensitivity to radiation is uniform, thereby providing an ideal surface for investigating the area-intensity relationship. The intensity of radiation necessary to evoke minimal sensation when various sized areas on the forehead and on the anterior body surface were exposed was measured. The data were found susceptible to analysis in three parts:

a. The portion of the curve for low intensities is interpreted to mean the *threshold of excitation*

of the most sensitive endings. This threshold is  $0.00015 \text{ cal/cm}^2/\text{sec}$ .

b. The portion of the curve for high intensities is assumed to mark the *threshold of sensation* of the sensitive end organs. This value was not carefully measured but lies between  $0.025$  and  $0.03 \text{ cal/cm}^2/\text{sec}$ .

c. The intermediate portion is a straight line obeying the formula:  $\log I + .78 \log A = -2.09$ . The similarity of the formula to the combined formulas for vision is pointed out.

7. The passage of impulses from end organs to the central nervous system without evoking sensation is demonstrated.

8. The importance of spatial summation on the magnitude of the sensory response is demonstrated. The present data are completely explained on the assumption that sensation intensity depends on the total frequency of impulses and upon summation.

9. The factors of summation and end organ distribution are shown to be highly variable for different parts of the body surface.

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# ACUTE MOUNTAIN SICKNESS; THE EFFECT OF AMMONIUM CHLORIDE<sup>1</sup>

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Our knowledge of the etiology of mountain sickness has not advanced very much since Paul Bert's fundamental observations (1), and little has been added to the understanding of its mechanism.

Detailed descriptions of the symptoms of mountain sickness have been given by those who have studied it, from Acosta in 1569 (2) to Monge et al. in 1928 (3) (Douglas, Haldane et al. (4), Barcroft et al. (5)). Although opinion on the fundamental cause of this disturbance is unanimous, there is disagreement as to the manner in which the organism endeavors to prevent the appearance of mountain sickness. Since this paper deals only with the disturbances produced as a consequence of rapid ascents to high altitudes, it will not discuss either Haldane's secretion theory, which has been shown to be incorrect by the Chilean studies of Dill, Christensen and Edwards (6), or Barcroft's claim (5) that the oxygen dissociation curve of hemoglobin deviates to the left, shown to be incorrect by Keys, Hall and Barron (7) and by Hall (8) on the same expedition.<sup>2</sup> By most observers the following have been considered processes of adaptation to high altitude: 1, a rise in the arterial oxygen pressure; 2, a fall in alveolar CO<sub>2</sub> pressure and a corresponding rise in alveolar oxygen pressure; 3, an increase in the per cent and total amount of the hemoglobin of the blood; 4, an acceleration of the blood flow. Some of these processes will be discussed in the light of our experience during an automobile trip from Lima (160 meters) to Ticio (4740 meters).<sup>3</sup>

<sup>1</sup> We wish to acknowledge our gratitude to Mr. Harold Kingsmill, General Manager of the Cerro de Paseo Mining Company, for providing facilities for the trip from Lima to Ticio.

<sup>2</sup> These authors give details of financial support and itinerary of the "International High Altitude Expedition."

<sup>3</sup> For rapidity of ascent from sea level to great alti-

## PROCEDURE

On the day preceding the trip to Ticio, six men (three medical students and three male nurses) of the twelve who made up the party took 15 grams each of NH<sub>4</sub>Cl in three 5-gram doses before breakfast, lunch, and dinner. On the following day, between 6:10 a.m. and 7:50 a.m., samples of alveolar air, taken at the end of normal expiration, and 50 cc. of arterial blood were obtained from each of these six individuals. The party started from Lima in three automobiles at 8:15 a.m., and after two halts (one in San Mateo (3200 meters) for one hour and another in Casapalca (4140 meters) for one and one-half hours) arrived at Ticio at 4:15 p.m. Upon their arrival the party had to walk about 200 meters to the engineer's house, where, after a rest of half an hour, arterial blood and alveolar air were taken from all but two members of the party. (Details concerning the individual reactions of the members of the party to the ascent are given at the end of this paper.)

## Arterial oxygen saturation

Since hemoglobin has been considered the main factor of oxygen transport in mammals, all investigators have given to its degree of oxygen saturation a rôle of prime importance in the causation of mountain sickness. Since there was no experimental evidence for this claim (no analysis of arterial blood having been performed), "cyanosis" and "blueness of the lips" were given as proof for their contention.

tudes few places in the world can compare with the Lima-Ticio region. It is for this reason peculiarly suited to the study of acute mountain sickness. This investigation is really an outcome of conversation on the Panama-Callao voyage with Dr. Crane, Chief Surgeon for the Cerro de Paseo Mining Company. His experiences make him a preeminent authority on the subject of acute mountain sickness.

TABLE I

Arterial oxygen saturation in acute mountain sickness. Arterial blood taken in Lima (160 meters) in the morning, before the trip, and in Ticlio (4740 meters) in the afternoon of the same day

Name	Lima		Ticlio		Condition
	HbO <sub>2</sub> content	HbO <sub>2</sub> saturation	HbO <sub>2</sub> content	HbO <sub>2</sub> saturation	
	mM. per liter	per cent	mM. per liter	per cent	
Sandoval.....	9.54	92.0	7.67	74.6	Ill. Mountain sickness
Aldazabar.....	9.71	92.6	7.49	70.3	Ill. Mountain sickness
Prieto.....			8.24	81.7	Ill. Mountain sickness
Cordova.....	8.24	92.7	6.44	72.9	Good
Tincopa.....	9.24	91.6	8.12	78.7	Good
Guzman.....	8.04	93.7	6.48	73.8	Good
A. Barron.....			6.17	65.4	Good
Hurtado.....			8.07	75.3	Good
E. S. G. Barron			8.05	73.3	Good
Average O <sub>2</sub> saturations in Ticlio:					
With mountain sickness.....					75.5
In good condition.....					73.2
Average O <sub>2</sub> saturation at Montt (4710 meters)—acclimatized members of the Chilean Expedition.....					78.0

In Table I are given the oxygen saturation and content of the arterial blood of the members of the party, as taken in Lima and in Ticlio. The average oxygen saturation of the individuals with mountain sickness was 75.5 per cent, while that of the individuals who did not feel any ill effects at this altitude was 73.2 per cent. Prieto, with an arterial oxygen saturation of 81.7 per cent, was ill; while A. Barron, with an oxygen saturation of 65.4 per cent, was well. Similar results were found earlier in Chile. In Montt, at 4710 meters, only 30 meters lower than Ticlio, Dill, with an arterial oxygen saturation of 71.6 per cent, was working with normal efficiency; while Hall, with an oxygen saturation of 83.2 per cent, had a mild case of mountain sickness (headache, lack of appetite, nausea).

#### Hemoglobin concentration

Barcroft (9), in his excellent studies on the physiology of the spleen, considers this organ as one of the main storehouses or depots for blood, adding as others the liver, lungs, and subpapillary vessels of the skin. The rôle of the spleen as a blood reservoir which the organism may utilize in case of need seems to vary in the different animal species. For example, while the dog's spleen, according to Barcroft, raises by its contraction during exercise the oxygen capacity of the blood,

such a rise does not occur in man (Dill, Talbott and Edwards (10)). It could be argued that during the ascent to high altitudes, before there is an increase in hemoglobin formation these blood reservoirs throw into the general circulation their reserve red cells, thus increasing the oxygen capacity of the blood. In Table II is given the oxygen capacity (a more reliable figure than the red cell count) of those members of the party whose arterial blood was obtained at both places, Lima and Ticlio. There was essentially no difference between the average arterial oxygen capacity in Lima, 9.68 mM. per liter, and that in Ticlio, 9.76 mM. per liter. Although there was a slightly higher average increase in the oxygen capacity of those who felt no ill symptoms than of those who suffered mountain sickness, the difference is too small to deserve attention. We may therefore conclude that these "blood stores" play no important function in the rapid adjustments of the organism to high altitudes. For comparison we have added the oxygen capacity of the acclimatized members of the Chilean Expedition after a gradual ascent to Montt, practically the same altitude as Ticlio. Here the increase in hemoglobin concentration is evident.

TABLE II

Arterial oxygen capacity soon after an ascent to high altitudes (Ticlio, 4740 meters)

Name	Oxygen capacity		Condition in Ticlio
	In Lima	In Ticlio	
	mM. per liter	mM. per liter	
Sandoval.....	10.37	10.25	Ill. Mountain sickness
Cordova.....	8.90	8.82	Good
Aldazabar.....	10.48	10.65	Ill. Mountain sickness
Tincopa.....	10.10	10.30	Good
Guzman.....	8.58	8.77	Good
Average.....	9.68	9.76	
Ill, mountain sickness.....	10.42	10.45	
In good condition..	9.19	9.30	
	Boston (sea level)	Montt (4710 meters)	
Members of the Chilean Expedition.....	8.84	10.72	



*Pressure of oxygen in alveolar air.*  
*Effect of  $\text{NH}_4\text{Cl}$*

A rise in the pressure of oxygen in the alveolar air, accomplished by an increase in the total ventilation, has been considered as one of the mechanisms of adaptation. Furthermore, Haldane and Priestley (11) state that "the diminution in available alkali seems to be much more important" for the process of adaptation to high altitudes. They then add, "Possibly this part of acclimatization might be greatly hastened by the administration of  $\text{NH}_4\text{Cl}$ " (11, p. 317). If an increase in the pressure of oxygen of the alveolar air may prevent mountain sickness, Haldane's suggestion seemed reasonable. Greene, during the expedition to Mount Kamet (12), took small doses of  $\text{NH}_4\text{Cl}$  (0.45 gram three times daily) and thought that the effect was beneficial. Later, Douglas, Greene and Kergin (13) compared the general condition and capacity to do muscular work of one subject at a pressure of 347 mm. Hg in a steel chamber with and without the administration of  $\text{NH}_4\text{Cl}$ . With  $\text{NH}_4\text{Cl}$  the subject showed a lower alveolar  $\text{CO}_2$  pressure and a higher oxygen pressure, a lessened degree of cyanosis, a slower pulse rate, and a greater ability to perform muscular work than in experiments in which no  $\text{NH}_4\text{Cl}$  had been taken. According to Fölling (14) the increase in alveolar ventilation after ingestion of  $\text{NH}_4\text{Cl}$  over a two-day period is not proportionate to the decrease in alkaline reserve, the result being an uncompensated acidosis.

The fact that there is no relation, *down to certain limits*, between the arterial oxygen saturation and mountain sickness raises strong doubts as to the usefulness of  $\text{NH}_4\text{Cl}$  in preventing mountain sickness. Furthermore, the uncompensated acidosis produced by the drug in displacing the oxygen dissociation curve to the right would tend to neutralize the effect of the increased oxygen pressure in the blood.

Notwithstanding these considerations, a practical test seemed worth while. This test was performed in Peru. Of the 12 members who made up the party, 6 took  $\text{NH}_4\text{Cl}$  in the doses stated under "Procedure."

Proof that this method of administering  $\text{NH}_4\text{Cl}$  resulted in a significant decrease in alkaline reserve and in alveolar ventilation over the critical

TABLE III

*Effects of  $\text{NH}_4\text{Cl}$  on blood and alveolar ventilation on normal subjects at sea level*

5 grams were ingested after each meal on the first day. Blood was drawn on the morning of the first day and 24 and 30 hours later. The results for the 3 specimens are in chronological order for each individual.

Subject	Alveolar air		Equilibrated oxygenated blood			Calculated arterial $\text{pH}_a$	Total $\text{CO}_2$ of oxygenated blood, when $\text{pCO}_2 = 40$ mm.
	$\text{pCO}_2$	$\text{pO}_2$	$\text{pCO}_2$	Total $\text{CO}_2$	$\text{HbO}_2$		
	mm. Hg	mm. Hg	mm. Hg	m.eq.	mM.		m.eq.
Dill.....	41.8	106	42.3	22.5	8.29	7.38	22.1
	38.3	110	42.1	18.7	8.38	7.33	18.3
	39.7	106	42.4	20.8	8.71	7.36	20.3
Forbes....	42.6	96	42.0	21.5	9.09	7.36	20.7
	40.2	103	40.9	18.2	8.80	7.30	18.0
	40.3	98	38.3	17.9	8.69	7.30	18.1
Edwards..	41.6	99	41.5	21.0	9.30	7.36	20.6
	38.0	103	39.6	17.2	9.58	7.30	17.2
	35.9	105	38.8	17.2	9.71	7.32	17.4
Keys.....	42.1	101	41.7	21.7	9.00	7.37	21.3
	38.8	107	38.4	17.4	8.90	7.30	17.7
	40.3	98	39.7	18.9	8.84	7.33	19.0
F. Con-solazio..	39.3	103	43.4	20.7	9.48	7.37	20.2
	34.5	109	37.4	16.3	9.71	7.32	17.0
			39.0	18.7	9.53		18.9
Daly.....	42.4	97	43.9	21.5	8.49	7.35	20.4
	35.2	120	37.7	16.7	9.26	7.32	17.3
			41.0	18.9	9.08		18.8
Mean values..	41.6	100	42.5	21.5	8.94	7.37	20.9
	37.5	109	39.4	17.4	9.10	7.31	17.6
	39.0	102	39.9	18.7	9.09	7.33	18.7

period was obtained after our return to Boston. Six subjects were given the same quantity at the same rate. Studies were made of the alveolar air and properties of the blood in the morning before beginning ingestion, the following morning, and the following afternoon. The results are shown in Table III. For comparison, the level of the  $\text{CO}_2$  curve of oxygenated blood of fully acclimatized men at various altitudes is shown in Table IV. It appears that the reduction in available alkali accomplished in 24 hours by the administration of  $\text{NH}_4\text{Cl}$  is approximately equal to that established by normal men after gradual (but incomplete) acclimatization to an altitude of about 5000 meters. Therefore, if alkaline reserve is closely related to mountain sickness, as has been claimed, one would expect significant benefits



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*tissue oxygen transport system* (myoglobin and part of the cytochrome complex), whose efficiency is regulated by the blood flow, the state of the capillaries, etc. The studies of Monge, Encinas, Hurtado and Heraud (3) and of Hurtado (17) on the dwellers of the high Andes, and those of the Chilean expedition, have shown that normal life is possible with an arterial oxygen saturation as low as 70 per cent. These studies suggest that undue importance has been given to the vascular oxygen transport system. Since mountain sickness may occur when the vascular oxygen transport system is still within normal limits, it is suggested that the tissue oxygen transport system plays an important rôle in determining the appearance of mountain sickness, because it contains and transports the molecular oxygen which will be immediately utilized by the oxidizing enzymes, i.e., the enzymes concerned with cellular respiration. Little is known of the properties of this system. However, Theorell's brilliant contribution (18) has shown that the oxygen dissociation constant of horse myoglobin at pH 7.46 is 3.26 mm.  $O_2$ ; i.e., the myoglobin, at equal hydrogen ion concentration and temperature, has six times as great an affinity for oxygen as horse hemoglobin has; and Millikan reports (19) that myoglobin combines with oxygen several times as fast as hemoglobin. This relation between the affinity for oxygen (as expressed by the dissociation constants) and the rate of combination in these two systems is of great physiological importance. The same relation between free energy and rates of reaction has been found by Barron (20) to exist in a number of oxidation processes of biological importance. The existence of cytochrome in the heart, liver, and brain of mammals has been reported by Cohen and Elvehjem (21). In sudden ruptures of equilibrium conditions (mountain sickness) this relation might influence the rate of diffusion of oxygen from the vascular oxygen transport system to the tissue oxygen transport system. Other factors which probably take part in the regulation of oxygen passage from the vascular system to the tissue transport system are an increased blood flow, as reported by Grollman (22), and dilatation of the capillaries.

## CONCLUSIONS

The appearance of acute mountain sickness is not closely dependent upon the degree of arterial oxygen saturation and the alveolar air oxygen pressure, down to certain limits. Acute mountain sickness is not prevented by diminishing the alkaline reserve of the blood, as shown by the failure of ammonium chloride to prevent it in a rapid ascent from sea level to 4740 meters. The tissue oxygen transport system (myoglobin and part of the cytochrome complex) probably plays an important rôle in mountain sickness.

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*Number 3. Aldazabar.* Nurse, 25 years. Born in Lima. Had been repeatedly at high altitudes. In Ticlio he felt extremely cold, had nausea, headache, dizziness, ringing in the ears. He was continuously shivering, so that samples of alveolar air could not be taken. He had taken  $NH_4Cl$ .

*Number 4. Prieto.* Student, 24 years. Born in Arequipa. Had never been in altitudes higher than Arequipa. From San Mateo on he felt headache and dizziness. In Casapalca he began to

TABLE IV  
CO<sub>2</sub> of oxygenated blood at pCO<sub>2</sub> = 40 mm.  
Results in m.eq. per liter

Name	Sea level	Chuquibambilla 2810 meters	Ollagüe 3660 meters	Montoya 4710 meters	Quelcheta 5340 meters	Punta 6140 meters
Members of expedition						
Forbes.....	22.4	19.4	17.6	19.3	17.3	16.2
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Dill.....	22.2	20.7		19.8	18.7	15.2*
Barron.....	20.9	19.2	18.9	18.7	19.2	15.1
Edwards.....	21.5	18.4		18.4	16.9	13.8*
Christensen.....	21.6	19.1	21.6	19.9	18.5	
Keys.....	22.3	18.2	19.4	17.4	17.2	15.7
McFarland.....	21.7	19.1		18.3	17.6	16.5
Hall.....	20.4	18.8		18.0	17.1	17.1
Matthews.....		19.2		19.3	17.5	16.8
Average...	21.7	19.1	19.2	18.8	17.8	16.2
Residents						
Bastias.....					16.2	
Heredia.....					14.8	
Campos.....					14.8	
Martinez.....			18.0			
Alcaino.....					17.4	
Troncoso.....					15.0	
Alcio.....					15.6	
Fritz.....					15.3	
Carrasco.....			18.3			
Average...					15.9	

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In Table V are given the alveolar pO<sub>2</sub> and pCO<sub>2</sub> of the members of the party from whom reliable samples were obtained. There was an increase in the alveolar pO<sub>2</sub> of those who took NH<sub>4</sub>Cl, and the average pO<sub>2</sub> was higher (49.6 mm. Hg) than those of Hurtado (45.0) and Barron (47.0), both thoroughly acclimatized to high altitudes. In spite of this increase in the pO<sub>2</sub> of the alveolar air, of the 6 subjects who took NH<sub>4</sub>Cl, one, Montoya, became critically ill at 4140 meters, and 2 more became ill in Ticlio; i.e., half of those who had taken NH<sub>4</sub>Cl had mountain sickness. Of the other 6 members, who had not taken NH<sub>4</sub>Cl, 2 had mountain sickness, one in Ticlio and the other on his way down. It should be emphasized that the three subjects who had taken NH<sub>4</sub>Cl and had mountain sickness had been in high altitudes previously with no ill effects. The gastric disturbances frequently produced by

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Although there is general agreement with Paul Bert's contention that the fundamental cause of mountain sickness is oxygen want, its mechanism is still obscure. In fact, Redfield's statement in 1922 (15) that in mountain sickness "each case is an individual story and up to the present no one has been able to predict who will and who will not be affected," as well as Loewy's remarks (16) made ten years later that "ihre Aetiologie jedoch ist durch neuste Beobachtungen eher dunkler geworden als aufgeklärt," may still be repeated.

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Since mountain sickness may be due to diminished oxygen utilization by certain tissues, in particular by the central nervous system, the factors concerned with the maintenance of a suitable oxygen supply to the tissues may be stated. These factors are essentially two: the *vascular oxygen transport system* (blood hemoglobin), whose efficiency is regulated by the arterial oxygen capacity and saturation and by the alveolar pO<sub>2</sub>; and the

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Cordova..	103.0	36.6	49.8	26.9	Yes	Good
Aldazabar..	104.0	35.6	50.9	24.8	Yes	Ill. Mountain sickness
Montoya..	103.9	34.9			Yes	Ill. Mountain sickness (Left in Casapalca)
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Prieto....					None	Ill. Mountain sickness
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*tissue oxygen transport system* (myoglobin and part of the cytochrome complex), whose efficiency is regulated by the blood flow, the state of the capillaries, etc. The studies of Monge, Encinas, Hurtado and Heraud (3) and of Hurtado (17) on the dwellers of the high Andes, and those of the Chilean expedition, have shown that normal life is possible with an arterial oxygen saturation as low as 70 per cent. These studies suggest that undue importance has been given to the vascular oxygen transport system. Since mountain sickness may occur when the vascular oxygen transport system is still within normal limits, it is suggested that the tissue oxygen transport system plays an important rôle in determining the appearance of mountain sickness, because it contains and transports the molecular oxygen which will be immediately utilized by the oxidizing enzymes, i.e., the enzymes concerned with cellular respiration. Little is known of the properties of this system. However, Theorell's brilliant contribution (18) has shown that the oxygen dissociation constant of horse myoglobin at pH 7.46 is 3.26 mm.  $O_2$ ; i.e., the myoglobin, at equal hydrogen ion concentration and temperature, has six times as great an affinity for oxygen as horse hemoglobin has; and Millikan reports (19) that myoglobin combines with oxygen several times as fast as hemoglobin. This relation between the affinity for oxygen (as expressed by the dissociation constants) and the rate of combination in these two systems is of great physiological importance. The same relation between free energy and rates of reaction has been found by Barron (20) to exist in a number of oxidation processes of biological importance. The existence of cytochrome in the heart, liver, and brain of mammals has been reported by Cohen and Elvehjem (21). In sudden ruptures of equilibrium conditions (mountain sickness) this relation might influence the rate of diffusion of oxygen from the vascular oxygen transport system to the tissue oxygen transport system. Other factors which probably take part in the regulation of oxygen passage from the vascular system to the tissue transport system are an increased blood flow, as reported by Grollman (22), and dilatation of the capillaries.

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*Number 4. Prieto.* Student, 24 years. Born in Arequipa. Had never been in altitudes higher than Arequipa. From San Mateo on he felt headache and dizziness. In Casapalca he began to

feel weak. At Ticlio, while walking to the engineer's house, he felt still weaker. At the house, he felt dizzy and faint; had nausea, vomiting, chills, extreme pallor, headache, and ringing of the ears. He was put to bed. His pulse was 88, rhythmic and vigorous. During the return to Lima, he began to feel ill again, at about 2100 meters, and vomited copiously. This was his first ascent to so high an altitude. He had taken no  $\text{NH}_4\text{Cl}$ .

*Number 5. A. G. Barron.* Member of the faculty in the Medical School of Lima, 33 years. Born in Huari. Extremely susceptible to mountain sickness. One week previously he had come by car along this road and had had to stop in Matucana (2370 meters) because of headache and dyspnea. He had had mountain sickness when passing through Ticlio by train on two previous occasions. In Ticlio he was feeling dyspneic and had tachycardia (pulse 128). On the way down, at about 2700 meters, he felt headache and nausea, and vomited. No blood sample could be taken. He had taken no  $\text{NH}_4\text{Cl}$ .

The other members of the party felt no ill symptoms; nor did the three drivers. Edwards, Hurtado and E. S. G. Barron worked steadily in Ticlio from 4:45 p.m. to 8:30 p.m., taking the blood and air samples. Edwards and Barron had recently been in the Chilean Andes, and Hurtado goes through Ticlio every other week to Morococha (4500 meters).

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# THE USE OF FERROUS GLUCONATE IN THE TREATMENT OF HYPOCHROMIC ANEMIA

By PAUL REZNIKOFF AND WALTHER F. GOEBEL

(From the New York Hospital and the Department of Medicine, Cornell University Medical College, and the Hospital of the Rockefeller Institute for Medical Research, New York City)

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Although hypochromic anemia due to iron deficiency is universally treated with iron medication, there is not as much unanimity with respect to the particular type of iron compound to be used. The following postulates, however, are accepted: The dosage must be adequate to insure a reasonably rapid increase of hemoglobin, the iron compound, in the dosage given, must be tolerated by the patient without undue distress, and the cost of medication must be within the financial means of the patient.

Many individuals receiving iron complain of such symptoms as nausea, epigastric discomfort, diarrhea or constipation. Some physicians hesitate to subject their patients to possible upset in cases of gastric or duodenal ulcer, colitis, and diarrhea or constipation. It was thought desirable, therefore, to prepare an iron compound which might have minimum irritating effects. One of the characteristics of ferric compounds is their ability to precipitate proteins. Most ferrous compounds are oxidized readily to the ferric state. This precipitating effect on proteins might explain the irritating action of iron compounds on the gastro-intestinal tract of some patients. Ferrous gluconate, prepared anaerobically, was found to have no precipitating action on proteins even when converted into the ferric state. It was decided, therefore, to treat patients with this compound to determine not only its efficacy but also its toxicity compared to medications now in common use. As a further test of its lack of toxic effects, solutions of ferrous gluconate were administered intramuscularly in quantities containing as much as 50 mgm. of iron, with no systemic disturbances and rarely with local discomfort. The method of preparing this substance and its use in treating hypochromic anemia in rats have been described previously (1).

Recently we have been able to simplify the method based upon a report of Neiger and Neuschul (2). These

investigators, studying the photochemical reactions of ferrous gluconate, prepared dilute solutions of this salt by boiling aqueous gluconic acid with iron filings. Although these workers do not describe the isolation of the crystalline salt, it occurred to us that crystalline ferrous gluconate might be prepared in quantity and in a high state of purity by employing this simple reaction. Consequently 100 grams of crystalline calcium gluconate<sup>1</sup> were dissolved in 700 cc. of boiling water. A solution of 29.3 grams of oxalic acid dissolved in 150 cc. of warm water was added. The precipitated calcium oxalate was separated by filtration, and the clear filtrate containing gluconic acid was concentrated to 350 cc. in vacuo. The solution of gluconic acid was placed in a one liter, three necked, round bottomed flask bearing a mercury sealed stirrer. One outlet of the flask was fitted with a small water trap to permit the escape of evolved hydrogen gas whereas the third outlet was closed with a rubber stopper. The flask was now heated in a water bath, and the contents rapidly stirred. Twenty-six grams (2 equivalents) of pure powdered iron (Merck's "Iron by Hydrogen") were added. A rapid evolution of hydrogen took place. At the end of two hours the solution in the flask was neutral to litmus paper. The hot solution of ferrous gluconate, colored a pale green, was carefully filtered through a sintered glass funnel of fine porosity. The filtration was conducted in such a manner that at no time did the solution of ferrous gluconate come in contact with atmospheric oxygen. This was accomplished by conducting the solution from the reaction flask by suction into an enclosed filtering system in which all air had been displaced by carbon dioxide.

The solution was allowed to cool in an atmosphere of carbon dioxide and after crystallization of ferrous gluconate was complete, the product was filtered rapidly in a Buchner funnel, washed with a small amount of 50 per cent alcohol, and finally with pure acetone. The substance was placed in a vacuum desiccator to remove all traces of acetone. Eighty-eight grams of ferrous gluconate were recovered.

The product thus obtained is a fluffy white powder with a very slight greenish tint. The substance crystallizes with one molecule of water and contains no detectable ferric iron. The fer-

<sup>1</sup> We have been able to simplify the preparation of ferrous gluconate still further recently by making it from technical gluconic acid.



TABLE I  
Summary of results with ferrous gluconate therapy in 13 patients

Case number	Diagnosis	Gastric HCl	Total quantity of iron given	Reticulocyte peak		Initial hemoglobin		Gain in hemoglobin under treatment		Time elapsed before increase in hemoglobin attained	Hemoglobin gain per day		Percentage utilization of iron	Remarks
				Per cent	Days after start of therapy	grams	per cent	grams	per cent		grams	per cent		
1. F. S.....	Hypochromic anemia; post-thyroidectomy; uterine fibroid	Hypochlorhydria	0.400* 2.160†	9.6	7	4.65 8.15	32 56	3.95 3.8	27 26	20 20	0.196 0.189	1.35 1.30	163.0 29.0	B. M. R. -3; menorrhagia continued; referred for hysterectomy
2. G. F.....	Hypochromic anemia; rheumatic heart disease	HCl present	0.225* 2.376†	10.2 9.4	5 4	8.60 10.20	59 70	1.9 2.5	13 17	7 18	0.270 0.138	1.86 0.95	139.0 17.4	Six months previously lost much blood from miscarriage
3. M. N....	Slight hypochromic anemia; rheumatic heart disease		0.156*	3.8	5	10.20	70	1.3	9	12	0.109	0.75	159.0	Convalescing from lobar pneumonia
4. E. S.....	Hypochromic anemia; rheumatic heart disease; uterine fibroid	Achlorhydria	0.475*	6.0	6	7.0	48	3.8	26	21	0.180	1.24	132.0	Hysterectomy after blood was normal
5. D. M....	Ulcerative colitis; ileostomy	Hypochlorhydria	0.400* 3.888† 4.288	22.0	21	7.5	52	6.7	46	55	0.122	0.84	25.8	High reticulocyte count at start of treatment; blood in stools; relapse when therapy stopped
6. M. G....	Duodenal ulcer; hematemesis		1.620†	15.2	2	9.8	67	4.1	28	16	0.251	1.75	41.0	Patient continued to show blood in stools constantly

TABLE I—Continued

Case number	Diagnosis	Gastric HCl	Total quantity of iron given	Reticulocyte peak		Initial hemoglobin		Gain in hemoglobin under treatment		Time elapsed before increase in hemoglobin attained	Hemoglobin gain per day		Percent-age utilization of iron	Remarks
				Per cent	Days after start of therapy	grams	per cent	grams	per cent		grams	per cent		
7. V. M....	Hypochromic anemia	Achlor-hydia	3.780†	9.0	9	5.7	39	8.6	59	38	0.225	1.55	37.5	Profuse menstruation during treatment. Menorrhagia persists after normal count and no medication
8. M. C....	Hypochromic anemia	Hypochlor-hydia	2.484†	6.6	4	8.0	55	5.7	39	23	0.250	1.70	37.9	Chief complaint headache which disappeared with normal count
9. M. M....	Hypochromic anemia; uterine fibroid	Achlor-hydia	4.212†	7.6	4	8.0	55	5.7	39	39	0.145	1.00	22.3	No drop in count after medication stopped in spite of menorrhagia. During experiment had profuse menstrual flow and upper respiratory infection. Hysterectomy
10. A. M....	Plummer-Vinson syndrome	Hypochlor-hydia	3.744†	6.0	9	8.6	59	3.2	22	40	0.080	0.55	14.1	Has developed a normocytic hypochromic anemia 4 months after normal count reached after faulty nutrition
11. L. V....	Hypochromic anemia	Achlor-hydia	2.592†	11.0	8	8.0	55	5.4	37	31	0.173	1.19	34.4	Failed to return to clinic for two three-week periods during study
12. C. DeV..	Hypochromic anemia	Achlor-hydia	3.456†	4.6	6	7.9	54	4.5	31	33	0.136	0.94	21.5	Thyroidectomy 3 years previously. B. M. R. +10 at present admission. Reticulocyte count 3.8 per cent at start of therapy
13. R. L. S...	Hypochromic anemia		3.672†	7.4	5	8.3	57	3.8	26	17	0.222	1.53	17.1	Intestinal adhesions

\* Intramuscular administration of ferrous gluconate.

† Oral administration of ferrous gluconate.

rous gluconate prepared in this manner is in all respects identical with that previously described. The above method, however, is considerably simpler than that originally described by us for the preparation of crystalline ferrous gluconate (1), and has the additional advantage of being more economical and more easily carried out.

Thirteen female patients ranging in age from 24 to 49 years and averaging 40, and suffering from hypochromic anemia were treated with ferrous gluconate, two by intramuscular injection, eight by oral administration and three by both methods (Table I). In addition, two patients who had demonstrated marked intolerance to other iron compounds were given ferrous gluconate. In most instances, daily reticulocyte, red blood cell and hemoglobin determinations were made until normal values were obtained, and subsequently the blood was studied as frequently as seemed indicated. In this study 14.5 grams of hemoglobin per 100 cc. was equivalent to 100 per cent.

The diagnosis in four of the patients was "idiopathic" hypochromic anemia without complicating factors. In the others, the following conditions were found, in some cases more than one being present: intestinal adhesions, 1; post-thyroidectomy, 2; uterine fibroids, 3; previous miscarriages, 1; rheumatic heart disease, 3; ulcerative colitis and ileostomy, 1; duodenal ulcer, 1.

The initial hemoglobin was less than 7.25 grams per 100 cc., or 50 per cent, in 3 of the patients. In the remaining, the hemoglobin before medication was greater than 7.25 grams. This observation is important since Heath (3) states that a 1 per cent rise in hemoglobin per day is the low limit of a satisfactory response to treatment when the initial hemoglobin is below 50 per cent.

The volume index of one patient who had been bleeding from a duodenal ulcer was 0.97. Another had a volume index of 0.8; in two the determination was not made; and in the other nine, the values varied from 0.58 to 0.76.

Gastric analysis was not performed in 3 cases. Of the rest, 1 had normal hydrochloric acid content after alcohol and histamine; 4 had hypochlorhydria; and 5, achlorhydria.

The anemia in all of these patients responded well to therapy. In only two did the hemoglobin level fail to reach 11.6 grams or 80 per cent and

in these the toxicity of ferrous gluconate when given intramuscularly was tested and no attempt was made to complete the treatment with this iron salt. Six of the 13 patients attained hemoglobin values ranging from 13.1 grams (90 per cent) to 14.2 grams (98 per cent); and 5, values ranging from 11.7 grams (81 per cent) to 12.6 (87 per cent).

Of the 13 patients, one had an initial erythrocyte count between 2,000,000 and 2,500,000; one, between 2,500,000 and 3,000,000; four, between 3,000,000 and 3,500,000; one, between 3,500,000 and 4,000,000; two, between 4,000,000 and 4,500,000; and four, between 4,500,000 and 5,000,000. After treatment, three patients had erythrocyte counts between 4,000,000 and 4,500,000; five, between 4,500,000 and 5,000,000; and five, above 5,000,000. Since this response of the red blood cells to treatment showed no abnormalities, this phase of the subject will not be considered further in this report.

Symptomatically, 7 patients were apparently cured with the attainment of a normal blood count. In 4, profuse menstrual bleeding persisted even after a normal blood count was reached. In 2 of these cases hysterectomy was performed (Cases 4 and 9); in the other two Cases 1 and 7), the blood count has been normal for five months in spite of the fact that they have continued to menstruate profusely and have received no medication. One patient (Case 5) who had an ileostomy for ulcerative colitis, became anemic again two months after the administration of ferrous gluconate was stopped although she received large doses of ferrous sulphate and intramuscular liver extract. Another patient (Case 10) returned three months after the cessation of ferrous gluconate therapy with evidences of hypochromic anemia and a history of severe malnutrition. She is responding well to liver therapy.

The effect of ferrous gluconate therapy is summarized in Table I. An analysis of these results, without a critical consideration of each case, shows that with respect to the reticulocyte count, gain in hemoglobin per day, and percentage utilization of iron, the ferrous gluconate was strikingly effective in the 13 patients treated. Since in this study small oral doses of iron (108 mgm. daily) were

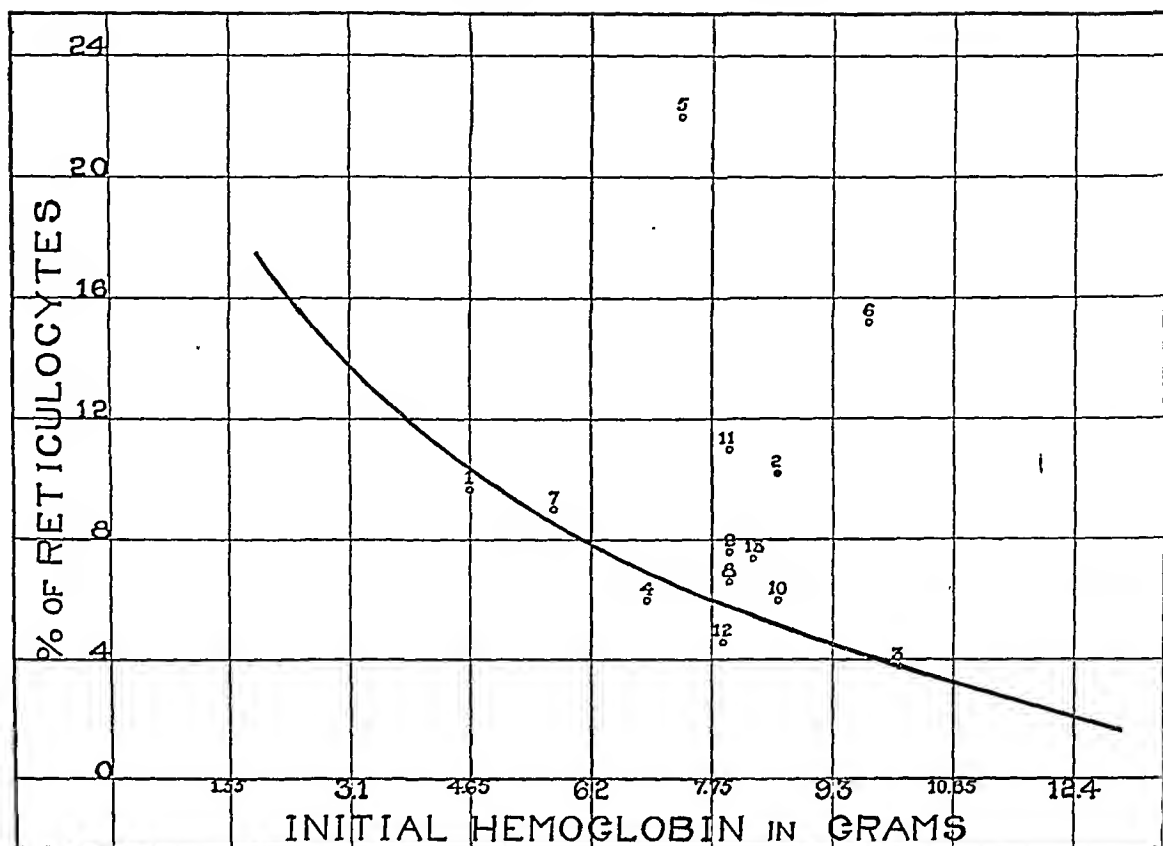


FIG. 1. RETICULOCYTE PEAKS ATTAINED BY PATIENTS PLOTTED ON HEATH'S (3) CURVE OF ADEQUATE RESPONSE

usually administered to determine the response to minimum dosage, the time elapsing before a normal blood count was obtained was not remarkable. However, when for purposes of expediency larger oral doses (216 mgm. daily) were given to Patients 2 and 13, normal blood counts were attained within three weeks. Patients 1, 6 and 8 showed a normal count with only 108 mgm. of iron daily in 20, 16 and 23 days respectively. Case 6, however, represents a posthemorrhagic patient, and the marked response may not be due chiefly to the therapy.

Figure 1 shows the reticulocyte peaks attained by the patients in this study. These are represented by a dot for each case when compared to Heath's curve (3) for adequate reticulocyte response. Patient 5 suffering from ulcerative colitis attained 22 per cent reticulocytes, but her initial count was 10 per cent. Patient 6 who suffered from hematemesis reached a peak of 15.2 per cent from an initial count of 12.6 per cent.

Obviously, these results cannot be considered to be entirely due to the treatment. Patient 12 attained a reticulocyte peak of only 4.6 per cent with an initial hemoglobin of 7.9 grams. But preliminary counts were as high as 2.4 per cent, and she had a red blood cell count of 4,800,000 before therapy was started which would tend to lower the peak.

The average daily increase of hemoglobin in the patients receiving ferrous gluconate intramuscularly, exclusive of Patient 5 in whom the results of intramuscular and oral administration overlapped, was 0.189 gram of 1.30 per cent. If Patient 3 is excluded because of her high initial count of 10.2 grams, the results with the remaining three patients, two of whom had initial hemoglobin values of less than 7.25 grams, give an average daily increase of hemoglobin for intramuscular therapy of 0.215 gram or 1.48 per cent. The average daily gain for all patients given ferrous gluconate orally was 0.181 gram or 1.25 per

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complained of indigestion characterized by anorexia, gas and epigastric distress or of constipation when receiving ferrous sulphate in quantities containing from 180 to 249 mgm. of iron per day.

### DISCUSSION

To evaluate the efficacy of ferrous gluconate, a comparison with results obtained with various iron compounds by other workers is necessary. However, it must be remembered that the experimental conditions may not be the same in any two studies. For example, Reimann and Fritsch (6) using ferrous chloride in doses which contained 100 mgm. of iron a day demonstrated remarkable utilization of the iron, ranging from 17 to 45 per cent. However, all their patients had other forms of iron until a few days before the ferrous chloride was started and some demonstrated excellent reticulocyte responses with these other iron compounds which were supposed to give no appreciable hemoglobin increase. It is obvious that the action of the alleged inactive iron salts cannot be disregarded in the final computations. In fact, these authors concluded that all ferrous compounds have approximately the same effect. It is also contrary to the experience of all other workers that large doses of ferric salts are ineffective in hypochromic anemia as Reimann and Fritsch assert. Schulten (7) found that ferrous chloride had to be given in much larger doses and saw no distinct advantage in this iron compound. Davidson (8) reported excellent results with ferrous chloride in doses containing 122 mgm. of iron a day but only 2 of his 7 patients attained 80 per cent hemoglobin. Witts (9) gives as the minimum effective daily dose of ferrous carbonate, an amount containing 300 mgm. of iron. Probably the best comparison of the efficacy of various iron compounds has been made by Fullerton (10). Table II represents a summary of his results compared with those obtained by oral administration of ferrous gluconate. Only those cases are included in which it seems reasonably certain that there are no factors which either interfere with or accentuate the iron effect. This control necessarily makes the available cases few. It is also important to compare separately the results in patients whose initial hemoglobin values were below and above 50 per cent. In our series, pa-

TABLE II  
*Relative efficacy of various iron compounds \**

Compound used	Daily iron dosage	Initial hemoglobin	Number of cases	Average daily hemoglobin rise	Average time before hemoglobin rise	Utilization of iron
	grams	per cent		per cent	days	per cent
Ferrous sulfate....	0.180	<50	12	1.175	30	15.70
Ferrous sulfate....	0.120	>50	3	0.650	33	13.00
Iron ammonium citrate.....	1.215	<50	30	1.270		2.50
Iron ammonium citrate.....	1.215	>50	3	1.030		2.03
Ferrous carbonate	0.110	<50	6	0.955		20.80
Ferrous carbonate	0.110	>50	3	0.520		11.30
Ferrous carbonate	0.220	<50	8	0.803		8.80
Ferrous carbonate	0.220	<50	1	0.180		1.96
Ferrous carbonate	0.330	<50	10	1.125		8.18
Ferrous carbonate	0.330	>50	3	0.940		6.84
Ferrous chloride..	0.132-0.198	<50	4	1.420		20.70
Ferrous chloride..	0.132-0.198	>50	3	1.000		14.50
Ferrous gluconate	0.108	<50	1	1.550	38	37.50
Ferrous gluconate	0.108	>50	5	1.230	29	29.00
Ferrous gluconate	0.216	>50	2	1.240	18	17.25

\* 14.5 grams = 100 per cent hemoglobin.

tients whose initial hemoglobin readings were below 7.25 grams are rare; in Fullerton's study initial hemoglobin values below 50 per cent were usual. In comparing the effect of the various iron salts it is important to note that all the patients receiving ferrous gluconate orally attained hemoglobin values greater than 11.6 grams or 80 per cent, while in Fullerton's series 9 of the 15 patients treated with ferrous sulphate and 2 of the 3 taking ferrous carbonate for whom data is given failed to reach such a level.

Since Barkan's (11) and Meulengracht's (12) reports, most clinicians feel that large doses of iron are essential in treating hypochromic anemia (13, 14, 15, 3, 16). Whipple and Robschey-Robbins (17), working with standard anemic dogs, emphasize the fact that the particular type of iron is unimportant as long as it is given in large doses. However, Fürth and Scholl (18) found that ferrous salts are much more easily absorbed from intestinal loops of rabbits than ferric compounds and most workers who advocate large doses of iron admit that ferrous salts are more efficiently utilized in patients (14, 3). Bethell, Goldhamer, Isaacs and Sturgis (19) feel the same about soluble iron salts. The results of most studies show, however, that large doses of iron may not produce a normal blood count for a considerable time (15) and occasionally rather small

cent. It is obviously unfair to include Patient 6 in this series as the rapid rise in her hemoglobin following the acute hemorrhage is certainly not due to medication entirely. Patient 2 whose initial hemoglobin before oral therapy was started was 10.2 grams and Patient 10 who subsequently demonstrated a maturative deficiency probably should not be considered in this calculation. If the last two patients are included, the average daily gain of hemoglobin for the group is 0.173 gram or 1.19 per cent; if these two cases are excluded, the average daily gain of hemoglobin is 0.191 gram or 1.32 per cent. Patient 7, the only one in the series whose initial hemoglobin was less than 7.25 grams before oral therapy was started, had a daily hemoglobin gain of 0.225 gram or 1.55 per cent.<sup>2</sup>

The percentage utilization of iron was calculated by multiplying the total gain in grams of hemoglobin per cubic centimeter for each patient by 5000, the approximate adult blood volume, and by 0.0033, the approximate percentage of iron in hemoglobin, and the result obtained was divided by the total amount of iron given the patient. The percentage utilization in the 4 patients who received intramuscular ferrous gluconate calculated in this manner was well over 100 per cent, actually averaging 148 per cent. This is in keeping with the findings of Heath, Strauss and Castle (4) and of Whipple and Robschey-Robbins (5). Whether this is due to the erroneous assumption of blood volume as the former authors suggest or to a salt effect on iron stored in the body as the latter workers state cannot be determined by our experiments. The percentage utilization of iron in the patients receiving ferrous gluconate by mouth averaged 27.2. If the three patients excluded from the final calculations of the daily hemoglobin increase are likewise omitted from this determination the utilization for oral administration of ferrous gluconate in doses ranging from 0.108 to 0.216 mgm. of iron daily is 28.5 per cent.

The problem of toxicity remains to be consid-

<sup>2</sup> After these cases were compiled, a patient was studied who had an initial hemoglobin of 5.7 grams or 39 per cent and was given daily doses of ferrous gluconate containing 324 mgm. Fe. In 11 days her hemoglobin rose to 11.6 grams or 80 per cent, a daily gain of 0.54 gram or 3.7 per cent.

ered. It is difficult to obtain objective evidence of intolerance to iron therapy. The following case, for example, which Dr. William Murphy of Boston kindly permits us to cite, illustrates an experience which is occasionally encountered. The patient had suffered from gastric and intestinal disturbances characterized by nausea, constipation and resulting hemorrhoids following the administration of various forms of iron. She also suffered from rectal irritation associated with nocturia, frequency and burning on urination. After taking 0.3 gram of ferrous gluconate three times a day, these symptoms were all produced but to a less degree than with other forms of iron which she tried. Dr. Murphy writes, "I am sure that she could take short courses with this form of iron with less difficulty than any of those which I have previously tried,—and I think it would be well for her to have these capsules for periodic use if it is possible to obtain them."

While this case is not very striking, a second patient offered a better means of studying the relative toxicity of ferrous gluconate. The patient in question was recovering from a Caesarian delivery and her obstetrician hesitated to give her iron for a slight anemia because she had suffered from gastro-intestinal distress and urticaria when she had received iron previously. At one time an injection of some iron compound had produced intense urticaria. When seen 17 days after her operation she had a red blood cell count of 3,600,000 and a hemoglobin of 11.7 grams or 80 per cent. She was not treated at the time but one and one-half months after discharge from the hospital she was given ferrous gluconate in increasing doses until she received 0.9 gram daily containing 108 mgm. of iron with no ill effects. She was then given 35 mgm. of iron in the form of ferrous sulphate and within a few hours suffered a violent gastro-intestinal upset. To determine the effect of the intramuscular injection of ferrous gluconate, she was given 0.45 cc. of a solution containing 25 mgm. of iron per cc. in her left gluteal muscle. For a few hours she had some swelling and soreness but no general reaction and the next morning the local region was practically normal.

At least two patients in this series who suffered no ill effects when taking ferrous gluconate in quantities containing 216 mgm. of iron per day,

complained of indigestion characterized by anorexia, gas and epigastric distress or of constipation when receiving ferrous sulphate in quantities containing from 180 to 249 mgm. of iron per day.

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Ferrous sulfate....	0.120	>50	3	0.650	33	13.00
Iron ammonium citrate.....	1.215	<50	30	1.270		2.50
Iron ammonium citrate.....	1.215	>50	3	1.030		2.03
Ferrous carbonate	0.110	<50	6	0.955		20.80
Ferrous carbonate	0.110	>50	3	0.520		11.30
Ferrous carbonate	0.220	<50	8	0.803		8.80
Ferrous carbonate	0.220	>50	1	0.180		1.96
Ferrous carbonate	0.330	<50	10	1.125		8.18
Ferrous carbonate	0.330	>50	3	0.940		6.84
Ferrous chloride..	0.132-0.198	<50	4	1.420		20.70
Ferrous chloride..	0.132-0.198	>50	3	1.000		14.50
Ferrous gluconate	0.108	<50	1	1.550	38	37.50
Ferrous gluconate	0.108	>50	5	1.230	29	29.00
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\* 14.5 grams = 100 per cent hemoglobin.

tients whose initial hemoglobin readings were below 7.25 grams are rare; in Fullerton's study initial hemoglobin values below 50 per cent were usual. In comparing the effect of the various iron salts it is important to note that all the patients receiving ferrous gluconate orally attained hemoglobin values greater than 11.6 grams or 80 per cent, while in Fullerton's series 9 of the 15 patients treated with ferrous sulphate and 2 of the 3 taking ferrous carbonate for whom data is given failed to reach such a level.

Since Barkan's (11) and Meulengracht's (12) reports, most clinicians feel that large doses of iron are essential in treating hypochromic anemia (13, 14, 15, 3, 16). Whipple and Robschey-Robbins (17), working with standard anemic dogs, emphasize the fact that the particular type of iron is unimportant as long as it is given in large doses. However, Fürth and Scholl (18) found that ferrous salts are much more easily absorbed from intestinal loops of rabbits than ferric compounds and most workers who advocate large doses of iron admit that ferrous salts are more efficiently utilized in patients (14, 3). Bethell, Goldhamer, Isaacs and Sturgis (19) feel the same about soluble iron salts. The results of most studies show, however, that large doses of iron may not produce a normal blood count for a considerable time (15) and occasionally rather small



cent. It is obviously unfair to include Patient 6 in this series as the rapid rise in her hemoglobin following the acute hemorrhage is certainly not due to medication entirely. Patient 2 whose initial hemoglobin before oral therapy was started was 10.2 grams and Patient 10 who subsequently demonstrated a maturative deficiency probably should not be considered in this calculation. If the last two patients are included, the average daily gain of hemoglobin for the group is 0.173 gram or 1.19 per cent; if these two cases are excluded, the average daily gain of hemoglobin is 0.191 gram or 1.32 per cent. Patient 7, the only one in the series whose initial hemoglobin was less than 7.25 grams before oral therapy was started, had a daily hemoglobin gain of 0.225 gram or 1.55 per cent.<sup>2</sup>

The percentage utilization of iron was calculated by multiplying the total gain in grams of hemoglobin per cubic centimeter for each patient by 5000, the approximate adult blood volume, and by 0.0033, the approximate percentage of iron in hemoglobin, and the result obtained was divided by the total amount of iron given the patient. The percentage utilization in the 4 patients who received intramuscular ferrous gluconate calculated in this manner was well over 100 per cent, actually averaging 148 per cent. This is in keeping with the findings of Heath, Strauss and Castle (4) and of Whipple and Robscheit-Robbins (5). Whether this is due to the erroneous assumption of blood volume as the former authors suggest or to a salt effect on iron stored in the body as the latter workers state cannot be determined by our experiments. The percentage utilization of iron in the patients receiving ferrous gluconate by mouth averaged 27.2. If the three patients excluded from the final calculations of the daily hemoglobin increase are likewise omitted from this determination the utilization for oral administration of ferrous gluconate in doses ranging from 0.108 to 0.216 mgm. of iron daily is 28.5 per cent.

The problem of toxicity remains to be consid-

<sup>2</sup> After these cases were compiled, a patient was studied who had an initial hemoglobin of 5.7 grams or 39 per cent and was given daily doses of ferrous gluconate containing 324 mgm. Fe. In 11 days her hemoglobin rose to 11.6 grams or 80 per cent, a daily gain of 0.54 gram or 3.7 per cent.

ered. It is difficult to obtain objective evidence of intolerance to iron therapy. The following case, for example, which Dr. William Murphy of Boston kindly permits us to cite, illustrates an experience which is occasionally encountered. The patient had suffered from gastric and intestinal disturbances characterized by nausea, constipation and resulting hemorrhoids following the administration of various forms of iron. She also suffered from rectal irritation associated with nocturia, frequency and burning on urination. After taking 0.3 gram of ferrous gluconate three times a day, these symptoms were all produced but to a less degree than with other forms of iron which she tried. Dr. Murphy writes, "I am sure that she could take short courses with this form of iron with less difficulty than any of those which I have previously tried,—and I think it would be well for her to have these capsules for periodic use if it is possible to obtain them."

While this case is not very striking, a second patient offered a better means of studying the relative toxicity of ferrous gluconate. The patient in question was recovering from a Caesarian delivery and her obstetrician hesitated to give her iron for a slight anemia because she had suffered from gastro-intestinal distress and urticaria when she had received iron previously. At one time an injection of some iron compound had produced intense urticaria. When seen 17 days after her operation she had a red blood cell count of 3,600,000 and a hemoglobin of 11.7 grams or 80 per cent. She was not treated at the time but one and one-half months after discharge from the hospital she was given ferrous gluconate in increasing doses until she received 0.9 gram daily containing 108 mgm. of iron with no ill effects. She was then given 35 mgm. of iron in the form of ferrous sulphate and within a few hours suffered a violent gastro-intestinal upset. To determine the effect of the intramuscular injection of ferrous gluconate, she was given 0.45 cc. of a solution containing 25 mgm. of iron per cc. in her left gluteal muscle. For a few hours she had some swelling and soreness but no general reaction and the next morning the local region was practically normal.

At least two patients in this series who suffered no ill effects when taking ferrous gluconate in quantities containing 216 mgm. of iron per day,

# RHEUMATIC FEVER AS A FAMILIAL DISEASE. ENVIRONMENT, COMMUNICABILITY AND HEREDITY IN THEIR RELATION TO THE OBSERVED FAMILIAL INCIDENCE OF THE DISEASE<sup>1,2</sup>

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The concentration of rheumatic fever in certain families, particularly the frequent occurrence of multiple cases in the same household, has long been recognized. The reported *familial* incidence of rheumatic fever, based on clinical studies, ranges from 15 to 58 per cent. Three factors have been implicated in the observed *familial* incidence of this disease. First, common environmental conditions; second, communicability; and third, susceptibility, probably on an hereditary basis. Up to the present time, conclusive evidence establishing any one or all of these hypotheses has not been presented.

It is believed that rheumatic fever has its highest incidence in urban populations of the north temperate zone. It also occurs in other sections of the world, particularly in localities where there are wide fluctuations in temperature. The disease appears to be more prevalent among the poorer classes of society, although not necessarily the poorest. The existence of unfavorable environmental conditions such as overcrowding, insufficient food and clothing, and frequent respiratory infections, are believed to be influential factors in the high *familial* incidence of the disease among the children of the poor. It does not, however, explain satisfactorily the occurrence of this disease in families of the well-to-do (1, 2, 3, 4, 5).

The simultaneous occurrence of rheumatic fever in several members of a family group has been reported. Epidemics of rheumatic fever have been described in barracks, boarding schools, convalescent homes, and hospital wards. These observations suggest that rheumatic fever may spread by intimate contact. It is generally be-

lieved that rheumatic fever is an infection exhibiting a low degree of contagiousness, in that not every exposed person contracts the disease. Paul (1) suggests that the disease spreads from one member of the household to another during, and in association with, respiratory infections.

Recently, emphasis is being placed by many observers on a peculiar susceptibility of the host to the disease. The identification of the susceptible or resistant individual is not possible by any known serological or cutaneous tests. Irvine-Jones (6) states that simultaneous attacks may be explained "by some nonspecific but infective agent attacking several people of like 'rheumatic' constitution." Draper (7) has suggested that rheumatic susceptibility may be a sex-linked character. It is of interest to note that Cheadle (8), in 1889, pointed out and emphasized that the tendency to rheumatism is transmitted, and many later observers have studied the hereditary transmission of the disease. A review of the literature, however, does not disclose adequate genetic analysis of sufficient data in conclusive support of this conception (9, 10).

The opportunity of observing a large number of rheumatic families over a period of years offered data which seemed suitable for an appraisal of the rôle of environment, contagion and heredity in their relation to the observed *familial* incidence of the disease. Material selected for study comprised 112 rheumatic families from the Children's Cardiac Clinic. Selection was based solely on residence in the lower and upper west and east sides of New York City, districts which could be adequately covered by two trained workers. These families, comprising 468 children over three years of age, equally divided as to sex, were under our observation from 3 to 18 years, an average of 9 years.

During the years 1933 to 1937, the homes were

<sup>1</sup> Presented before the Cornell Research Society, March 8, 1937.

<sup>2</sup> This study was conducted under a special grant from the Commonwealth Fund.

doses of iron will cause surprising improvement (20).

The real problem of iron therapy is not the theoretical utilization of iron, or the reticulocyte response, or even the daily increase of hemoglobin for any particular period of treatment. These are important only as they indicate the return of the patients' blood to normal in a reasonably short time without undue inconvenience. Most patients suffering from hypochromic anemia respond well to most forms of iron when administered in adequate dosage. For the patients who cannot tolerate the usual iron compounds, it is important to have a medication which is effective and which causes minimum disturbance. For all patients in need of iron it is desirable to use a compound which gives good results with the least discomfort. Ferrous gluconate seems to be such a medicament.

#### CONCLUSIONS

1. Ferrous gluconate prepared in the absence of oxygen has been used in the treatment of 13 patients suffering from hypochromic anemia.

2. The use of ferrous gluconate compared with other iron preparations results in satisfactory reticulocyte responses, a high percentage utilization of iron, and such daily increase in hemoglobin that a normal level occurs in a reasonably short time.

3. Four patients, who showed toxic reactions to other iron compounds, were able to take ferrous gluconate without any undue distress.

4. In the patients who received ferrous gluconate intramuscularly up to the present no systemic and only rare and mild local reactions occurred. However, in view of the efficacy of the oral administration of ferrous gluconate and its lack of toxicity there is seldom any reason for its parenteral administration.

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TABLE I  
Age and sex distribution of siblings in 112 families

Total births	Under 3 years		Siblings				Sex distribution			
	Dead	Living	4 to 6 years		7 to 15 years and over		Total siblings		Rheumatic siblings	
			Rheu- matic	Nor- mal	Rheu- matic	Nor- mal	M.	F.	M.	F.
509	19	22	8	43	219	198	240	228	104	123
			Total 468				51	49	46	54
			Total rheumatics 227				per cent	per cent	per cent	per cent

under very close observation; visits were made weekly or semi-monthly. A family record was kept of the onset and termination of every illness of each member of the household, including all respiratory infections. The environmental conditions in the homes were noted, i.e., available budget, size of the family, number of rooms, light, heat, dampness, food habits, clothing, and maternal care. With few exceptions, physical and fluoroscopic examinations were made of every member of the family at the Clinic.<sup>3</sup> In some instances, death certificates, hospital records and statements from physicians were accepted. An historical pedigree of each family was obtained as accurately as was possible. The diagnosis of rheumatic fever, active or inactive, and of rheumatic heart disease, was made according to the criteria of the Heart Committee of the New York Tuberculosis and Health Association, Inc. (11).

The family was taken as the unit for study and a graphic record was kept for each family (Figure 2), similar to that used by McPhedran and Opie (12) in their studies on tuberculosis in families.

#### *Environmental factors in their relation to the familial incidence of rheumatic fever*

The families studied were of varying economic levels—ranging from dire poverty to middle class comfort. Rheumatic families from the higher income brackets were not represented in this series.

<sup>3</sup> We wish to acknowledge our indebtedness to Dr. E. Ingeman, Dr. R. O. DuBois, Dr. B. McL. Spock, Dr. Betty Huse, C. Lingg, R. Johnstone, L. Ward, and E. Colt for their cooperation in these studies.

The wage earners in 77 per cent of the families were unskilled laborers; in 23 per cent of the families they were white collar workers.

From close observation of the existing home conditions it was determined that in about one-third of the families, Group 1, there was an adequate budget, food was sufficient in quantity and well balanced, rooms were dry and sunny, clothing was ample and maternal care sensible. In the remaining two-thirds, comprising Groups 2, 3, and 4, certain conditions were observed which could be considered as possible predisposing factors to the disease. In Group 2 (13 per cent), rooms were dark and damp, and clothing was inadequate. In Group 3 (30 per cent), the food was insufficient in quantity and poorly balanced. In Group 4 (24 per cent), rooms were dark and damp, clothing was inadequate, food was insufficient in quantity and poorly balanced, and maternal care was poor.

A comparative analysis of the incidence of rheumatic siblings in these four environmental groupings, reveals them to be remarkably comparable. In Group 1, the incidence was 53 per cent; in Group 2, it was 45 per cent; in Group 3, it was 38 per cent; in Group 4, it was 54 per cent.

These findings parallel those reported from the London Hospitals (4), namely, that incidence of rheumatic siblings in Class A (comparable to Group 1) was 16 per cent; in Class B (com-

TABLE II  
Relation of environment to incidence of rheumatic fever in 112 families \*

Parental groupings	Total families		Environmental groups†							
			Group 1		Group 2		Group 3		Group 4	
	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent
Parents —	57	100	17	30	9	15.7	18	31.5	13	22.8
Mother +	36	100	15	42	3	8.3	10	27.7	8	22.0
Father +	15	100	3	20	3	20.0	5	33.3	4	26.6
M + and F +.....	4	100	2	50					2	50.0
Total.....	112	100	37	33	15	13.4	33	29.4	27	24.1

\* Social economic status: Unskilled labor 77 per cent. White collar 23 per cent.

† Environmental Groups:

- 1: Adequate budget, food sufficient and well balanced, dry sunny rooms, ample clothing, sensible maternal care.
- 2: Rooms dark and damp, clothing inadequate.
- 3: Insufficient food, poorly balanced.
- 4: Rooms dark and damp, clothing inadequate, insufficient food, poorly balanced, maternal care poor.

TABLE 111

Incidence of rheumatic siblings in relation to environment and heredity

Parental group	Total number of families	Environmental status											
		Favorable				Unfavorable							
		Group 1			Group 2		Group 3		Group 4				
		T.S.	R. S.		T.S.	R. S.	T.S.	R. S.		T.S.	R. S.		
			per cent			per cent			per cent			per cent	
Parents —...	57	70	29	41.4	30	11	36.6	77	28	36.3	63	25	39.0
Mother +...	36	51	34	66.6	10	4	40.0	43	16	37.2	43	29	67.4
Father +....	15	20	10	50.0	7	6	85.7	16	8	50.0	20	14	70.0
M + and F +	4	7	6	85.7							11	7	63.0
Total positive families...	55	78	50	64.1	17	10	58.8	59	21	40.0	74	50	67.5
Total families...	112	148	79	53.3	47	21	44.6	136	52	38.2	137	75	54.0
		T. S.: 320      R. S.: 148    (46.2 per cent)											

Key:

T. S.: Total siblings.

R. S.: Rheumatic siblings.

(+): Rheumatic, or positive.

(-): Non-rheumatic, or negative.

Pos. Fam.: Positive families, includes mother +, father + or both parents +.

parable to Groups 2 and 3), it was 12 per cent; and in Class C (comparable to Group 4), it was 10 per cent.

If the conditions concomitant with dire poverty were the determining factors influencing the familial incidence of the disease, one might have expected the highest incidence in Group 4 (our series) and Class C (London Hospitals).

It is apparent from these observations that within the economic level represented by these families (which excludes families of the well-to-do), there was no direct relation between the incidence of rheumatic siblings and the environmental status.

A comparison of the incidence of rheumatic siblings in the various environmental classes, in relation to the presence of positive (rheumatic) parents, indicates that the incidence of rheumatic siblings is greater in each series with parental rheumatism (Figure 1). In Group 1, for example, where environmental conditions were favorable, the incidence of rheumatic siblings of negative (non-rheumatic) parents was 41 per cent, as compared with an incidence of 64 per cent within

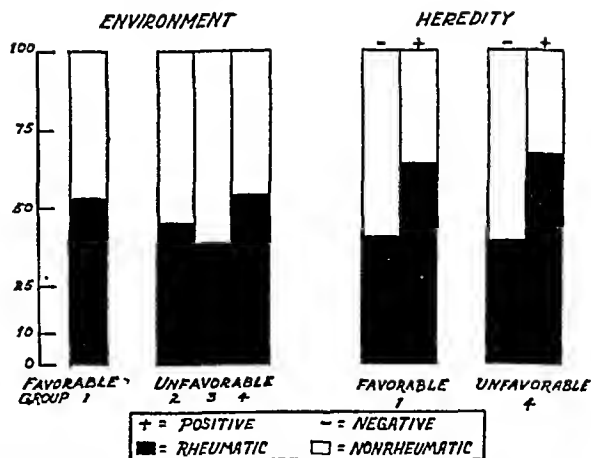


FIG. 1. INCIDENCE OF RHEUMATIC SIBLINGS IN RELATION TO ENVIRONMENT AND HEREDITY

the same environmental group but of rheumatic parents. Similarly, in Group 4, where unfavorable environmental conditions were present, the incidence was 39 per cent, compared with 67 per cent. A comparative analysis of the incidence of rheumatic siblings in relation to environment and to parental rheumatism in families of only four or five siblings, showed a similar trend.

### Comment

In view of the high incidence of rheumatic fever among under-privileged children and the low incidence among children of the well-to-do, it might be expected that the *familial* incidence of the disease would be in direct relation to the type of environment. The data presented, however, reveals the incidence of rheumatic fever to be comparable in the various environmental groups studied. Comparative data on the *familial* incidence of rheumatic fever among rheumatic families of a higher economic level were not available for comparison. Should such studies reveal a low *familial* incidence it would indicate that some common environmental factor was operative in families of lower economic levels.

It is recognized, however, that unfavorable environmental conditions tend to increase the incidence of any infection. It cannot, therefore, be concluded that unfavorable environmental conditions are not contributing factors in this disease. However, the data presented would indicate that unfavorable environmental conditions are not the determining factor responsible for the observed

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509	19	22	Total 468 Total rheumatics 227				51 per cent	49 per cent	46 per cent	54 per cent

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These findings parallel those reported from the London Hospitals (4), namely, that incidence of rheumatic siblings in Class A (comparable to Group 1) was 16 per cent; in Class B (com-

TABLE II

*Relation of environment to incidence of rheumatic fever in 112 families \**

Parental groupings	Total families		Environmental groupings†							
			Group 1		Group 2		Group 3		Group 4	
	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent
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Father +	15	100	3	20	3	20.0	5	33.3	4	26.6
M + and F +.....	4	100	2	50					2	50.0
Total.....	112	100	37	33	15	13.4	33	29.4	27	24.1

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*Incidence of rheumatic siblings in relation to environment and heredity*

Parental group	Total number of families	Environmental status													
		Favorable				Unfavorable									
		Group 1				Group 2		Group 3		Group 4					
		T. S.		R. S.		T. S.		R. S.		T. S.		R. S.			
Parents —...	57	70	29	41.4	36	11	36.6	77	28	36.3	63	25	39.0		
Mother +...	36	51	34	66.6	10	4	40.0	43	16	37.2	43	29	67.4		
Father +...	15	20	10	50.0	7	6	85.7	16	8	50.0	20	14	70.0		
M + and F +	4	7	6	85.7							11	7	63.6		
Total positive families...	55	78	50	64.1	17	10	58.8	59	24	40.6	74	50	67.5		
Total families...	112	148	79	53.3	47	21	44.6	136	52	38.2	137	75	54.0		
												T. S.: 320		R. S.: 148 (46.2 per cent)	

T. S.: Total siblings.  
R. S.: Rheumatic siblings.  
(+): Rheumatic, or positive.  
(-): Non-rheumatic, or negative.  
Pos. Fam.: Positive families, includes mother +, father  
+ or both parents +.

**ENVIRONMENT**

Group	Rheumatic (%)	Nonrheumatic (%)
FAVORABLE GROUP 1	55	45
UNFAVORABLE 2	45	55
UNFAVORABLE 3	40	60
UNFAVORABLE +	55	45

**HEREDITY**

Group	Rheumatic (%)	Nonrheumatic (%)
FAVORABLE -	42	58
FAVORABLE +	65	35
UNFAVORABLE -	40	60
UNFAVORABLE +	68	32

**Legend:**  
 + = POSITIVE      - = NEGATIVE  
 ■ = RHEUMATIC      □ = NONRHEUMATIC

It is recognized, however, that unfavorable environmental conditions tend to increase the incidence of any infection. It cannot, therefore, be concluded that unfavorable environmental conditions are not contributing factors in this disease. However, the data presented would indicate that unfavorable environmental conditions are not the determining factor responsible for the observed



familial incidence of the disease in this group. The significant increased incidence of rheumatic siblings that was observed in the series with parental rheumatism compared with the offspring of negative parents, admits of two interpretations: either that the parents may have been a source of exposure, or that heredity may be an important factor.

### *Communicability of rheumatic fever in its relation to the familial incidence of the disease*

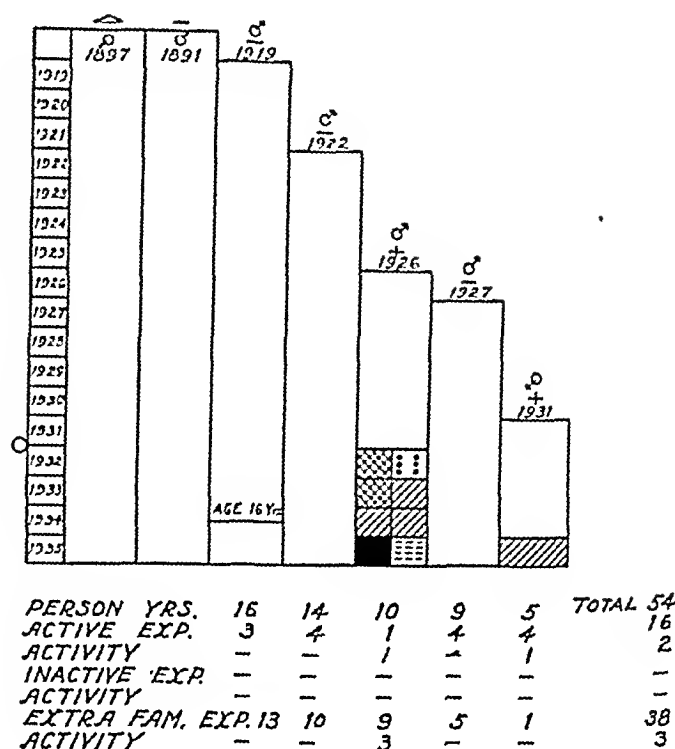
If rheumatic fever is an infection which may be conveyed by contagion, its incidence to various types and sources of exposure may be determined. The method of procedure which seemed suitable for obtaining this information is similar to that used by Opie et al. in their studies on the spread of tuberculosis in families (12).

The family graph included the record of every member of the household starting from the year of birth of the oldest sibling through the year 1935. The average period of observation of these families was 9 years—ranging from 3 to 18 years. The onset and recurrence of rheumatic episodes (joint pains, polyarthritides, chorea, carditis and nodules) were recorded for every individual for each calendar year. Parental rheumatic activity occurring before the birth of the first sibling was not tabulated.

The biometric method utilized for the analysis was the "person year" method, that is, the unit was one person observed for one year.

To test the theory that rheumatic fever may be spread in families living in close contact, certain possible methods of spread were analyzed.

1. A rheumatic member of a family experiencing manifestations of rheumatic activity in any part of the calendar year might constitute a source of exposure to the rest of the household. This was arbitrarily termed a *person year of "active exposure."* (It may be argued that this method of analysis is not valid for the reason that contagiousness may exist only during the period of a preceding event such as a respiratory infection, the "primary phase" described by Coburn (13). However, since the person year unit would necessarily represent all rheumatic activity preceded by respiratory infections, it actually serves as a basis of estimating contagiousness no matter in what



GRAPH "V"

FIG. 2. FAMILY GRAPH, "V" AND "L"

Includes mother and father and 5 siblings. Parents negative although there was a history of rheumatism on maternal side (mother's sister had rheumatic heart disease).

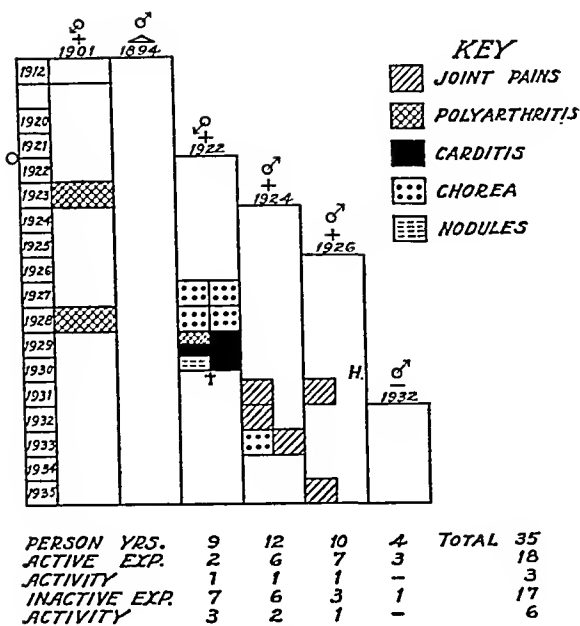
*Sibling 1* had a total of 16 person years, during 13 of which there was no exposure in the family. For 3 years there was "active exposure" to Sibling 3, with no resulting activity.

*Sibling 2* had a total of 14 person years, of which 10 years were years of "extra-familial exposure" and 4 were years of "active exposure" (3 years to Sibling 3 and 1 year to both Sibling 3 and Sibling 5) with no resulting activity.

*Sibling 3* (Primary rheumatic in family—onset 1932) had a total of 10 person years, of which 9 were years of no exposure within the family. Patient manifested rheumatic activity after 6 years of "extra-familial exposure," resulting in 3 years of rheumatic activity. The 4th year of activity was simultaneous with activity in Sibling 5, and was considered a year of "active exposure" to Sibling 5.

*Sibling 4* had a total of 9 person years, 5 of which were years of no familial exposure and 4 were years of "active exposure" (3 to Sibling 3 and 1 to Siblings 3 and 5), with no resulting activity.

*Sibling 5* had a total of 5 person years. There was one year of "extra-familial exposure" and 4 years of "active exposure" to Sibling 3. After 3 years of "active exposure" to Sibling 3, patient developed rheumatic activity during the 4th year of exposure. The manifestation was "joint pains" which occurred during the same year as carditis and nodules in Sibling 3 (after an interval of 3 months).



GRAPH "L"

FIG. 2. FAMILY GRAPH, "V" AND "L"

Includes positive mother, negative father (whose sister had chorea as child), 3 positive siblings and 1 negative sibling. Mother had polyarthritides and chorea in childhood. In 1923 and 1928, mother had polyarthritides. For two years she was a source of "active exposure" to the remainder of the family and for 14 years she was a source of "inactive exposure."

*Sibling 1* had a total of 9 person years, dying of rheumatic carditis in 1930. Person years comprised 2 years of "active exposure" and 7 years of "inactive exposure." Total of 4 years of activity. Onset of rheumatism was 4 years after "active exposure" to mother. (Prior to "active exposure," there was 1 year of "inactive exposure" 1922.) Following the year of "active exposure" (1923) there were 3 years of "inactive exposure" before the onset in 1927. During 1928, activity was simultaneous with activity in the mother.

*Sibling 2.* Total of 12 person years, of which 6 were years of "active exposure" and 6 were years of "inactive exposure." Onset of rheumatism in 1931 was preceded by 3 years of "inactive exposure," followed by 4 years of "active exposure." Activity occurred one year after "active exposure" to Sibling 1 and simultaneously with activity in Sibling 3.

*Sibling 3.* Total of 10 person years, of which 7 were years of "active exposure" and 3 of "inactive exposure." Onset was preceded by 1 year of "inactive exposure" and 4 years of "active exposure," occurring during simultaneous year of activity in Sibling 2.

*Sibling 4.* Total of 4 person years, three of which were years of "active exposure," resulting in no activity. One year was a year of "inactive exposure," resulting in no activity.

period of rheumatic activity such contagiousness may exist. Respiratory infections occurring in December, with subsequent rheumatic activity in January, might conceivably be excluded. Checking on our data for 79 families for the calendar year of 1933 (14), we found only 3 rheumatic recurrences out of a total of 139 that would have been excluded in this way. If one were to assume further that every rheumatic episode has its contagious phase in a preceding respiratory infection, our maximum error from this same source is but 10 per cent for this year.)

2. Rheumatic individuals experiencing intercurrent years of apparent freedom from rheumatic activity, might still be a possible source of exposure. Such years were calculated as *person years of "inactive exposure."*

3. The primary case in a family may be considered to have acquired the disease by casual contact outside the family. The years prior to the onset of rheumatic activity were arbitrarily termed *person years of "extra-familial exposure."*

The total person years of "active" and "inactive" exposure, as well as the total person years of "extra-familial exposure" in each family, were calculated for each member of the household. Since rheumatic activity manifests itself mainly in childhood (from 4 to 16 years of age), the data tabulated for analysis included only person years of *children* reaching the ages of 4 to 16 years. (The application of the method is illustrated in the two family graphs: Figure 2, "V" and "L.") The interval elapsing between exposure and onset of the disease was calculated for both "active" and "inactive" exposure. Where "inactive exposure" preceded "active exposure," both were noted. Individuals having simultaneous years of activity were arbitrarily considered to have exposed each other.

It was possible to obtain more accurate data on the time relationship between exposure and onset of activity by an analysis of the simultaneous years of activity in these families for a period of three calendar years, when the dates of onset and termination of each rheumatic episode were observed and recorded.

In Table IV is presented a comparative analysis of the incidence of rheumatic fever in the 112 families, in relation to the source and type of exposure. Here it may be seen that for 55 fam-

familial incidence of the disease in this group. The significant increased incidence of rheumatic siblings that was observed in the series with parental rheumatism compared with the offspring of negative parents, admits of two interpretations: either that the parents may have been a source of exposure, or that heredity may be an important factor.

#### *Communicability of rheumatic fever in its relation to the familial incidence of the disease*

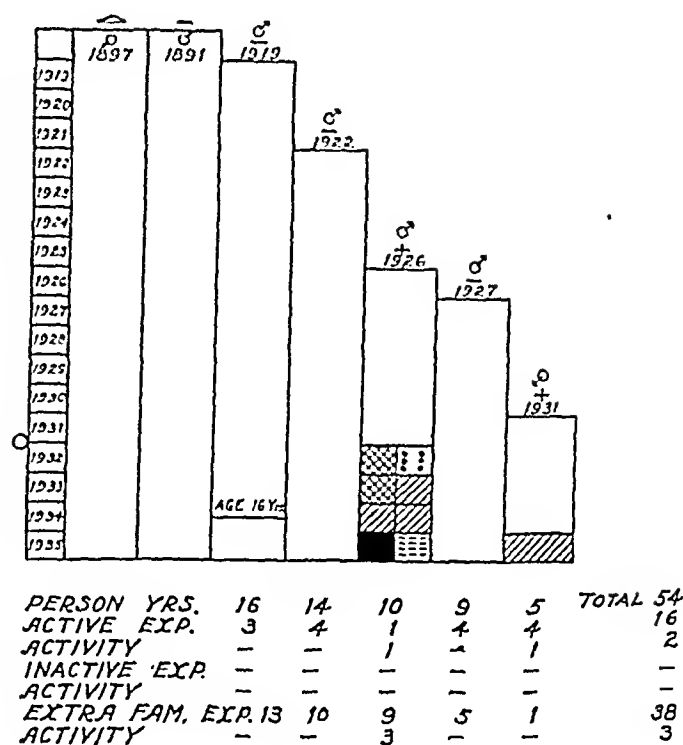
If rheumatic fever is an infection which may be conveyed by contagion, its incidence to various types and sources of exposure may be determined. The method of procedure which seemed suitable for obtaining this information is similar to that used by Opie et al. in their studies on the spread of tuberculosis in families (12).

The family graph included the record of every member of the household starting from the year of birth of the oldest sibling through the year 1935. The average period of observation of these families was 9 years—ranging from 3 to 18 years. The onset and recurrence of rheumatic episodes (joint pains, polyarthritides, chorea, carditis and nodules) were recorded for every individual for each calendar year. Parental rheumatic activity occurring before the birth of the first sibling was not tabulated.

The biometric method utilized for the analysis was the "person year" method, that is, the unit was one person observed for one year.

To test the theory that rheumatic fever may be spread in families living in close contact, certain possible methods of spread were analyzed.

1. A rheumatic member of a family experiencing manifestations of rheumatic activity in any part of the calendar year might constitute a source of exposure to the rest of the household. This was arbitrarily termed a *person year* of "active exposure." (It may be argued that this method of analysis is not valid for the reason that contagiousness may exist only during the period of a preceding event such as a respiratory infection, the "primary phase" described by Coburn (13). However, since the person year unit would necessarily represent all rheumatic activity preceded by respiratory infections, it actually serves as a basis of estimating contagiousness no matter in what



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Includes mother and father and 5 siblings. Parents negative although there was a history of rheumatism on maternal side (mother's sister had rheumatic heart disease).

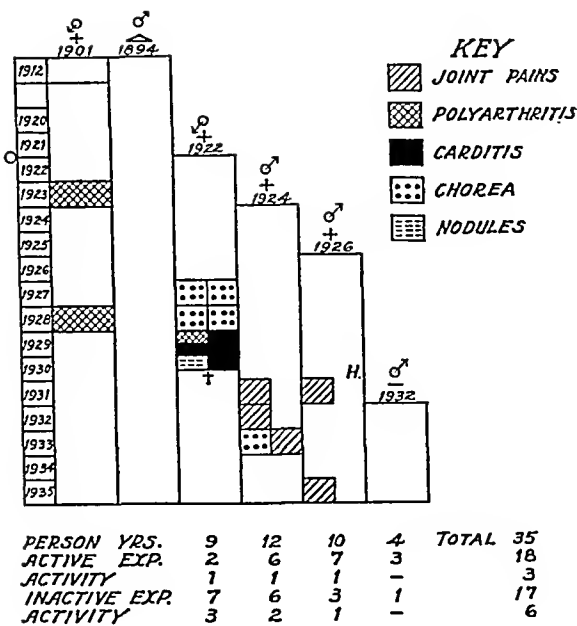
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It was possible to obtain more accurate data on the time relationship between exposure and onset of activity by an analysis of the simultaneous years of activity in these families for a period of three calendar years, when the dates of onset and termination of each rheumatic episode were observed and recorded.

In Table IV is presented a comparative analysis of the incidence of rheumatic fever in the 112 families, in relation to the source and type of exposure. Here it may be seen that for 55 fam-

TABLE IV

*Incidence of rheumatic fever in its relation to source and type of exposure*

	Mother +		Father +		M + and F +		Total + parents		Total - parents	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Number of families (112).....	36		15		4		55		57	
Number of siblings (468).....	147		63		18		228		240	
Number of rheumatic siblings (227).....	83	56.4	38	60.3	13	72.2	134	58.6	93	38.7
Total person years.....	1832		748		223		2803		2955	
Person years "Active exposure".....	598	32.6	284	38.0	86	38.5	968	34.5	713	24.0
Person years "Inactive exposure".....	1234	67.3	464	62.0	137	61.4	1835	65.4	569	19.0
Person years "Extrafamilial exposure".....									1673	57.0
Total years "Active exposure" result. Activity	114	19.0	51	17.9	35	40.6	200	20.6	90	12.6
Total years "Inactive exposure" result. Activity	156	12.6	59	12.7	9	6.5	224	12.2	60	10.5
Total years "Extra-familial exposure" result. Activity									196	11.7
									1673	
Total person years active.....	270		110		44		424		346	
To "Active exposure".....	114	42.2	51	46.3	35	79.5	200	47.1	90	26.0
To "Inactive exposure".....	156	57.7	59	53.6	9	20.5	224	52.8	60	17.3
To "Extra-familial exposure".....									196	56.6

ilies with parental rheumatism, giving an opportunity for familial exposure, of a total of 2,803 person years 35 per cent were years of "active exposure" and 65 per cent were years of "inactive exposure." It is of interest that only 21 per cent of the years of "active exposure" in these families could be considered to have resulted in rheumatic fever. One hundred and thirty-four rheumatic siblings experienced 424 person years of activity; 47 per cent of these years of activity occurring subsequent to "active

exposure," and 53 per cent followed "inactive exposure." It is apparent that "active" and "inactive" exposure were equally effective.

In a comparison of the incidence of rheumatic fever in the 57 families with negative parents, an opportunity for comparing the relation of the incidence of rheumatic fever to the source of exposure (that is, familial or extra-familial) was offered. Of a total of 2,955 person years, 57 per cent were years of "extra-familial exposure," and 43 per cent were years of "familial exposure"

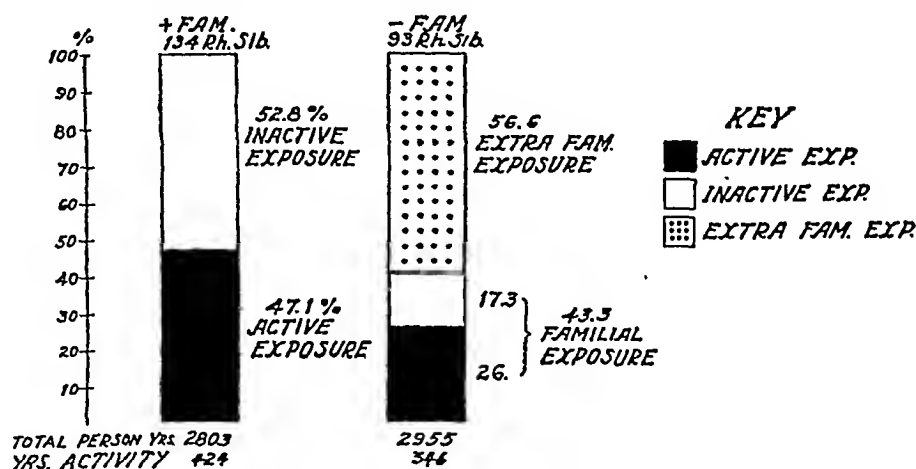


FIG. 3. TOTAL PERSON YEARS OF ACTIVITY RELATED TO SOURCE AND TYPE OF EXPOSURE

(24 per cent of which were years of "active exposure," and 19 per cent "inactive exposure"). "Familial exposure" resulted in 11.8 per cent of activity, as compared with 11.7 per cent following "extra-familial exposure." Ninety-three rheumatic siblings experienced 346 person years of activity, of which about one-half (57 per cent) followed "extra-familial exposure" (Figure 3).

There is another method of demonstrating the relative influence of "extra-familial exposure" (Table V). In 53 of the families with negative

TABLE V  
*Frequency of primary and secondary rheumatic siblings of negative parents*

	Total	Positive	Percentage positive
Second or later sibling where oldest is negative.....	48	20	42 ± 7
Second or later sibling where oldest is positive *.....	65	28	43 ± 6
Second or later sibling where oldest is positive †.....	35	16	46 ± 8
Incidence among firstborn ‡.....	53	24	45 ± 7

\* Including only those born before first sibling became positive.

† Including only those born after first sibling became positive.

‡ Excluding one sibling families, and those families where first sibling became positive after a later sibling was positive.

(non-rheumatic) parents, a division was made depending on whether the first child was negative or positive. Where the first child was negative, 42 per cent of 48 subsequent siblings were rheumatic. Where the first sibling was positive, 44 per cent of 100 subsequent siblings were rheumatic. The incidence of rheumatism among the firstborn, as given in the table, was 42 per cent. The relative incidence of rheumatic fever is, therefore, the same for primary and secondary cases in a family. These observations indicate that exposure was as effective from casual contact ("extra-familial exposure"), as it was by intimate contact within the family.

An attempt was made to determine the time relationship between the type of exposure and the onset of the disease. In Table VI it may be seen that in only 20 per cent was the onset of the disease within a year of "active exposure." In 49 per cent the onset was within 2 to 5 years, and in 31 per cent the onset was within 6 to 15 years.

TABLE VI  
*Interval between exposure and onset*

Interval between familial exposure and onset of rheumatic activity	Active exposure		Inactive exposure	
	Number	Per cent	Number	Per cent
1 year.....	24	20	15	14
2 to 5 years.....	58	49	63	57
6 to 15 years.....	37	31	32	29

There was no significant difference in the time intervening between the onset of the disease after "active" and "inactive" exposure.

The occurrence of rheumatic activity in more than one member of a family during the same year suggests communicability of the disease. Of a total of 588 calendar years of activity, 159, or 27 per cent, were simultaneous years. The interval between exposure and onset of various types of rheumatic activity were observed and recorded. In Table VII, and Figures 4 and 5,

TABLE VII  
*Type of rheumatic manifestation and interval between simultaneous calendar years of activity \**

Related manifestations	Total manifestations		Interval					
			1 month		1 to 2 months		2 to 11 months	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Joint pains and joint pains....	69	33.0	29	42	17	24	23	34
Joint pains and polyarthritis or carditis.....	47	22.5	16	34	12	24	19	42
Joint pains and chorea.....	48	23.0	8	16	15	31	25	53
Polyarthritis, carditis and chorea.....	45	21.5	8	18	7	16	30	66
Total related manifestations.	209	100	61	29.1	51	24.4	97	46.4

\* The onset and termination of every illness was recorded for 112 families over a period of three years.

it may be seen that in only 29 per cent was the interval one month; in 24 per cent it was 1 to 2 months; in 47 per cent it was 2 to 11 months. Approximately three-fourths of the related manifestations were between joint pains and some other manifestation of the disease, and in only one-fourth were related major manifestations, i.e., polyarthritis, carditis and chorea. In this group, in 66 per cent the interval between exposure and activity was 2 to 11 months.

It is of interest that in 51 families, 59 parents (mother or father) were rheumatic; 27, or 44

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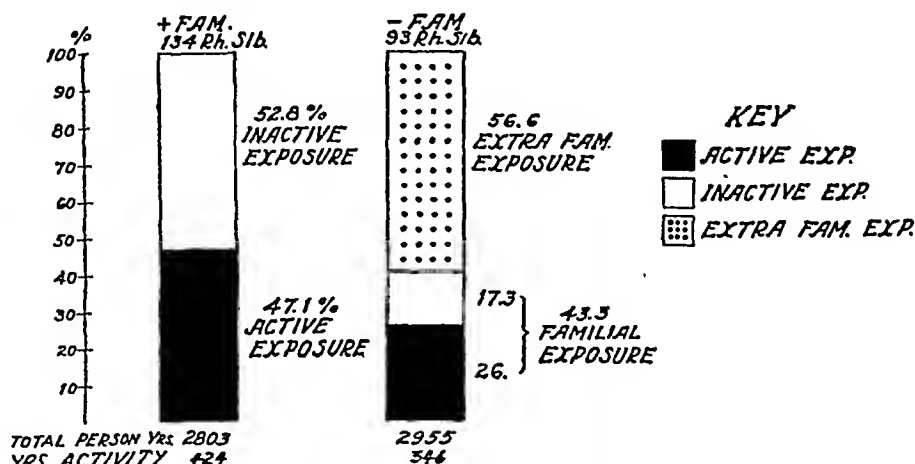


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It is of interest that in 51 families, 59 parents (mother or father) were rheumatic; 27, or 44



per cent, of the parents experienced rheumatic activity during the lifetime of the siblings. In no instance did a negative parent acquire the disease.

the household respiratory infections could be related to recurrence of rheumatic activity. Rheumatic children experienced more frequent respiratory infections than non-rheumatic children in

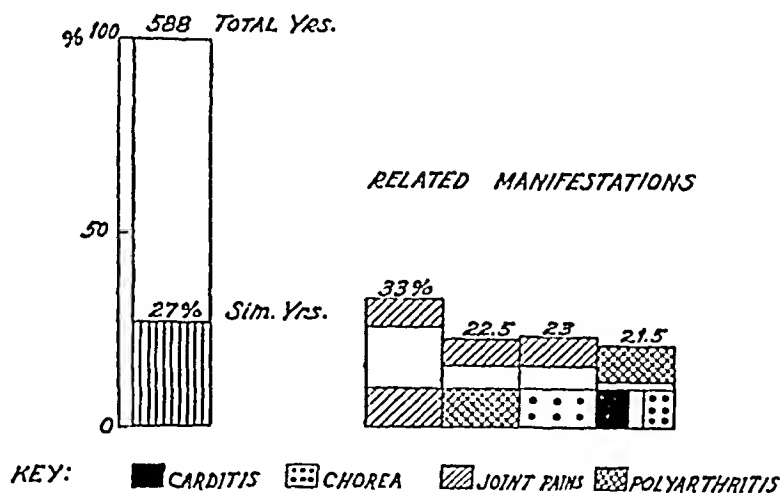


FIG. 4. SIMULTANEOUS YEARS OF ACTIVITY

The average age of onset of rheumatic fever in the negative and positive families was 7 years of age; the earliest age of onset was 2 years. During the period of observation, of 123 non-rheumatic siblings ranging in age from 15 to 30 years, no brother or sister negative to the age of 15 years, subsequently acquired the disease.

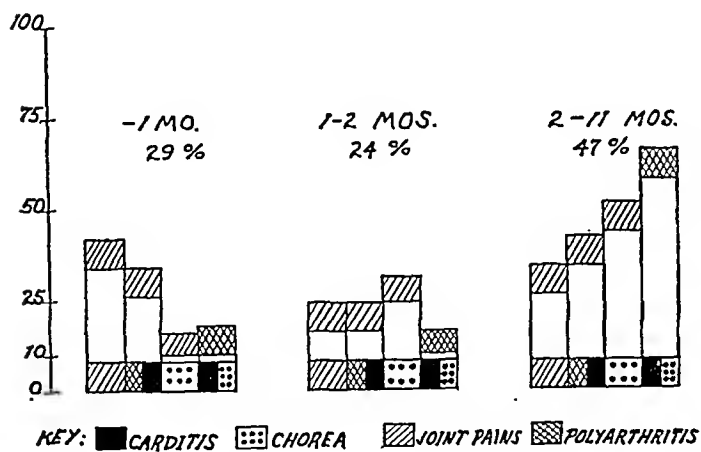


FIG. 5. INTERVAL BETWEEN ASSOCIATED MANIFESTATIONS—SIMULTANEOUS YEARS

Studies on the relationship between respiratory infections and the occurrence of rheumatic fever in these families have been published (14). Continued close supervision of these families for the past two years confirmed the observations reported. The occurrence of respiratory infections in the families were not associated with the spread of rheumatic activity. Less than 15 per cent of

these families. The majority of the respiratory infections occurred simultaneously with, during, or following rheumatic activity. During the periods 1930 to 1932 and 1933 to 1937, respiratory infections were observed to precede only 10 to 25 per cent of rheumatic recurrences (within a period of 3 to 4 weeks). These observations do not indicate that there is a causal relationship between respiratory infections and the occurrence of rheumatic fever in the families studied. If rheumatic fever is communicable during a preceding or associated respiratory infection, there should have been a difference between the effectiveness of "active" and "inactive" exposure.

It is evident from the data presented that there is no direct relation between the type and source of exposure and the resulting disease. Some factor, other than intimate contact, must be primarily responsible for the observed familial incidence of the disease in these families.

#### Comment

It might be expected that if rheumatic fever is spread in the families by intimate contact, there would be evident a direct relation between "familial exposure" and infection. The data secured, however, indicate that there is no apparent relation between type and source of exposure and resulting disease. There was no significant difference in the incidence of the disease following "active" or "inactive" exposure.

The incidence of the disease following intimate contact ("familial exposure") and casual contact ("extra-familial exposure") were comparable. Within the limits of our accuracy of clinical diagnosis and method of analysis utilized, it is evident that the duration of the interval between exposure and the onset of the disease is not like that of other contagious diseases. A consideration of all the data secured would indicate that if rheumatic fever is spread in families by contagion, the acquisition of the disease is primarily determined by some factor other than exposure to a rheumatic individual. That the factor may be one of hereditary susceptibility is suggested by the increased incidence of rheumatic siblings in series of subjects with parental rheumatism.

*Heredity in its relation to the observed familial incidence of rheumatic fever*

It has already been suggested that there is a direct relation between the incidence of rheumatic siblings and parental rheumatism. The reciprocal of this relation was also found to hold. Inquiry into the family pedigree of these patients disclosed that in 55 families one or both parents were rheumatic. In addition, in 29 families, or 53 per cent, of this group—grandparents, aunts, uncles or cousins were rheumatic. In 57 families both parents were negative, but of this group, 26 families (46 per cent) had rheumatic individuals on the maternal or paternal sides. In only 28 per cent of the 112 families were we unable to obtain a definite history of rheumatism. These results suggest the operation of genetic factors.

The data adequate for genetic analysis included 122 families which had been examined in the Clinic. (Four of the 112 used in the preceding part of the paper were omitted, and twelve others added.) In addition, 273 consecutive cases of rheumatic patients from the pediatric cardiac clinic were used. The information on familial incidence was obtained from the clinic charts, on which this information was routinely recorded, and, unlike the 122 families intensively studied, it does not represent examination of all the members of the family. From the literature, additional data for analysis was obtained from the British Medical Research Council Report (3), and from Draper (7). Irvine-Jones' data on twins (6) were used.

The incidence of rheumatic fever among twins may also be used as a criterion of the importance of genetic factors in the familial incidence of this disease. It will be recalled that identical twins are descended from a single ovum and sperm, while fraternal twins are derived from two independent fertilized eggs. If genetic factors play a rôle in the occurrence of rheumatic fever, it would be expected that differences would be found between identical and fraternal twins. In our records there were seven pairs of twins, of which two pairs were identical. Dr. Irvine-Jones has published data on nine additional pairs. These are included in Table VIII. In this table,

TABLE VIII  
*Incidence of rheumatic fever in twins*

	Identical				Fraternal			
	Total	Pairs			Total	Pairs		
		++	--	+-		++	--	+-
Original data....	2	2	0	0	5	2	1	2
Irvine-Jones (7).	2	2	0	0	7	0	2	5
Total.....	4	4			12	2	3	7

of the four sets of identical twins, all four are alike in having rheumatic fever. Of the twelve pairs of fraternal twins, five are similar (that is, either both have rheumatic fever or both are free from it) and seven pairs are dissimilar. Inasmuch as the environmental features associated with all twins are usually comparable, it is difficult to explain this different incidence in fraternal and identical twins. They are, however, fully consistent with a genetic interpretation which requires that identical twins be similar, while fraternal twins would not be expected to be any more alike than any two siblings.

Recent progress in genetic science has made it possible to approach problems of human heredity in relation to disease with greater precision than has hitherto been possible.

In the application of Mendelian formulae to data on human heredity, it is necessary to introduce certain corrections for the inadvertent selection inherent in the original data. It will be recalled that the families selected for study were drawn from the children in attendance at the

per cent, of the parents experienced rheumatic activity during the lifetime of the siblings. In no instance did a negative parent acquire the disease.

the household respiratory infections could be related to recurrence of rheumatic activity. Rheumatic children experienced more frequent respiratory infections than non-rheumatic children in

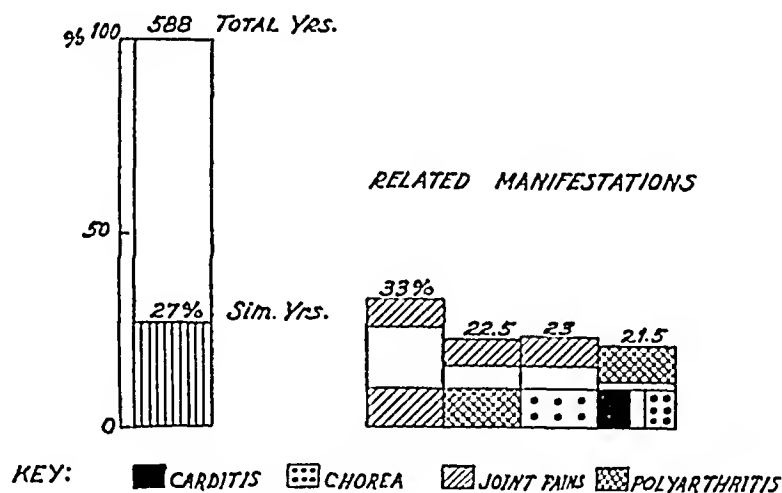


FIG. 4. SIMULTANEOUS YEARS OF ACTIVITY

The average age of onset of rheumatic fever in the negative and positive families was 7 years of age; the earliest age of onset was 2 years. During the period of observation, of 123 non-rheumatic siblings ranging in age from 15 to 30 years, no brother or sister negative to the age of 15 years, subsequently acquired the disease.

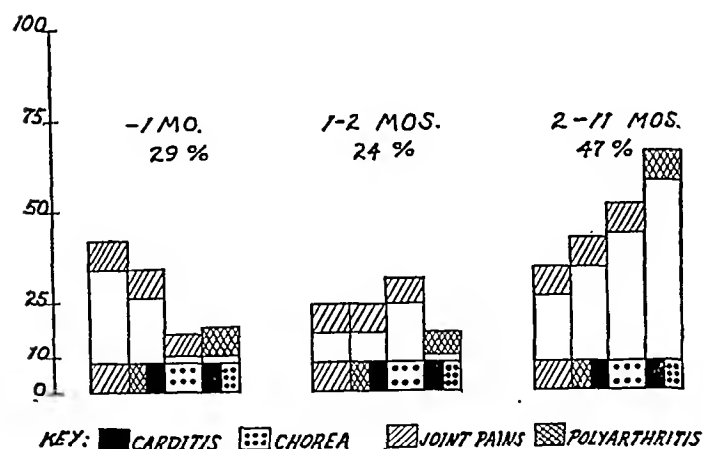


FIG. 5. INTERVAL BETWEEN ASSOCIATED MANIFESTATIONS—SIMULTANEOUS YEARS

Studies on the relationship between respiratory infections and the occurrence of rheumatic fever in these families have been published (14). Continued close supervision of these families for the past two years confirmed the observations reported. The occurrence of respiratory infections in the families were not associated with the spread of rheumatic activity. Less than 15 per cent of

these families. The majority of the respiratory infections occurred simultaneously with, during, or following rheumatic activity. During the periods 1930 to 1932 and 1933 to 1937, respiratory infections were observed to precede only 10 to 25 per cent of rheumatic recurrences (within a period of 3 to 4 weeks). These observations do not indicate that there is a causal relationship between respiratory infections and the occurrence of rheumatic fever in the families studied. If rheumatic fever is communicable during a preceding or associated respiratory infection, there should have been a difference between the effectiveness of "active" and "inactive" exposure.

It is evident from the data presented that there is no direct relation between the type and source of exposure and the resulting disease. Some factor, other than intimate contact, must be primarily responsible for the observed *familial* incidence of the disease in these families.

#### Comment

It might be expected that if rheumatic fever is spread in the families by intimate contact, there would be evident a direct relation between "familial exposure" and infection. The data secured, however, indicate that there is no apparent relation between type and source of exposure and resulting disease. There was no significant difference in the incidence of the disease following "active" or "inactive" exposure.

The incidence of the disease following intimate contact ("familial exposure") and casual contact ("extra-familial exposure") were comparable. Within the limits of our accuracy of clinical diagnosis and method of analysis utilized, it is evident that the duration of the interval between exposure and the onset of the disease is not like that of other contagious diseases. A consideration of all the data secured would indicate that if rheumatic fever is spread in families by contagion, the acquisition of the disease is primarily determined by some factor other than exposure to a rheumatic individual. That the factor may be one of hereditary susceptibility is suggested by the increased incidence of rheumatic siblings in series of subjects with parental rheumatism.

*Heredity in its relation to the observed familial incidence of rheumatic fever*

It has already been suggested that there is a direct relation between the incidence of rheumatic siblings and parental rheumatism. The reciprocal of this relation was also found to hold. Inquiry into the family pedigree of these patients disclosed that in 55 families one or both parents were rheumatic. In addition, in 29 families, or 53 per cent, of this group—grandparents, aunts, uncles or cousins were rheumatic. In 57 families both parents were negative, but of this group, 26 families (46 per cent) had rheumatic individuals on the maternal or paternal sides. In only 28 per cent of the 112 families were we unable to obtain a definite history of rheumatism. These results suggest the operation of genetic factors.

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TABLE IX

Comparison of observed incidence of rheumatic siblings with expectation from Mendelian formulae

Number of siblings in each family	Number of families	Observed number of rheumatic siblings	Expected number of rheumatic siblings	Difference	$\sigma$	Difference/ $\sigma$
A. NEGATIVE FEMALE $\times$ NEGATIVE MALE						
1	1	1	1			
2	11	12	12.6	0.6	1.35	0.5
3	12	17	15.6	1.4	3.16	0.8
4	13	21	19.0	2.0	5.42	0.9
5	9	17	14.7	2.3	5.32	1.0
6	4	10	7.3	2.7	3.00	1.6
7	3	9	6.1	2.9	2.91	1.7
8	3	5	6.7	1.7	3.54	0.9
9	1	1	2.4	1.4	1.38	1.2
10	1	1	2.6	1.6	1.59	1.3
Total	58	94	88.0		27.72	
				1.1 $\sigma$	=5.25	
B. POSITIVE FEMALE $\times$ NEGATIVE MALE						
1	2	2	2			
2	6	10	8	2	1.31	1.7
3	8	14	13.7	0.3	3.91	0.2
4	7	16	14.9	1.1	5.48	0.5
5	3	7	7.7	0.7	3.24	0.4
6	4	11	12.2	1.2	5.52	0.5
7	3	11	10.6	0.4	5.00	0.2
8	3	14	12.0	2.0	5.83	0.8
9	1	5	4.5	0.5	2.22	0.3
Total	37	90	85.6		32.51	
				0.7 $\sigma$	=5.8	
C. NEGATIVE FEMALE $\times$ POSITIVE MALE						
1	1	1	1			
2	2	2	2.7	0.7	0.44	1.0
3	3	6	5.1	0.9	1.47	0.7
4	3	7	6.4	0.6	2.34	2.5
5	2	6	5.2	0.8	2.16	1.8
6	1	1	3.0	2.0	1.38	0.6
7						
8						
9	1	6	4.5	1.5	2.22	1.0
Total	13	29	27.9		10.01	
				0.3 $\sigma$	=3.2	

Cardiac Clinic. Consequently, every family in the group contained at least one rheumatic child. Since this would not be true of a random sample of the population, a correction has to be introduced. It may be noted here that the correction must be made separately for each size of family. The details of the statistical method have recently

been reviewed by Hogben (15) and Levit (16).

There is presented in Table IX (A, B, and C), a comparison of the observed and expected values for each family of similar size. In crosses of 58 families of negative parents, the observed incidence was 94, compared with an expected value of 88. For 37 families with positive mothers and for 13 families with positive fathers, the observed and expected were respectively, 90 compared with 86, and 29 compared with 27. As shown in the table, the differences between observed and expected are clearly not statistically significant in any of these instances.

In analyzing the mechanism of inheritance involved in this disease, there are five major possibilities which will be considered: simple dominance; a single autosomal recessive; two or more recessive factors; two or more dominant factors; and sex linkage.

*Simple dominance.* Any heredity character which is inherited as a simple dominant should be expressed in every generation of a line in which the factor is being transmitted. Inasmuch as 48 per cent of the 122 families have negative parents, this possibility may be excluded. The subsequent discussion of penetrance indicates that *partial dominance* may also be excluded.

*Single recessive and two or more recessive genes.* If more than one recessive gene are necessary for the expression of a given trait, the proportion of families with more than one member affected should be fewer the larger the number of factors involved (Hogben (15)). Table X shows the comparison of the observed inci-

TABLE X

Comparison of observed incidence of families with more than one rheumatic sibling with expectation from single recessive and double recessive gene hypotheses

NEGATIVE FEMALE $\times$ NEGATIVE MALE					
Number of siblings in each family	Number of families	Families having more than 1+	Expected double recessive hypothesis	Observed	Expected single recessive hypothesis
			per cent	per cent	per cent
1	1	0	0	0	0
2	11	1	3	9	15
3	12	4	6	33	27
4	13	6	9	41	39
5	9	7	13	78	48
6	4	2	15	50	56
7	3	2	18	67	64

dence of multiple cases with the expected values if one or two recessive factors were involved. For the case of four siblings, the number expected for a single recessive case is 39 per cent; for a double recessive case it is 9 per cent. The actual value, 41 per cent, is in much closer agreement with the expectation for a single factor. The same agreement with single factor requirements also holds for all other sizes of families. Predictions for three and four recessive factors were computed and found to be even less in agreement with the observations than is the two factor hypothesis.

*Single recessive and two or three dominant genes.* The analysis for the hypotheses of two, three or four dominant factors in comparison with a single recessive factor, gives different predicted values for each hypothesis.

The computations in Table XI permit the com-

TABLE XI

*Comparison of observed incidence of rheumatism in families with expectation from single recessive, double dominant and triple dominant gene hypotheses*

NEGATIVE FEMALE X NEGATIVE MALE

Number of siblings in each family	Number of families	Observed single	Expected single recessive hypothesis	Expected double dominant hypothesis	Expected triple dominant hypothesis
1	1	1	1	1	1
2	6	6	7.8	6.4	6.2
3	14	18	18.2	15.9	14.9
4	13	18	19.0	15.7	14.3
5	8	17	13.1	10.3	9.1
6	3	6	5.5	4.1	4.5
7	1	5	2.0	1.4	1.2
8	3	5	6.7	4.6	3.7
9	1	1	2.4	1.6	1.3
Total	50	77	75.7	61.0	56.2

parison between the incidence of rheumatic diseases observed in the progeny of negative parents, with the values predicted from a single recessive gene, and from two dominant or three dominant genes. The observed frequency, 77, is in closer agreement with the predicted value from one recessive gene 75.7; than it is with two dominant genes 61.0; or three dominant genes 56.2. Computations for more than three dominant factors were still more divergent from the observations.

*Sex linkage.* A test for sex linkage was also made. Among the progeny of positive mothers by negative fathers, 58 per cent of 71 daughters

were positive and 51 per cent of 78 sons were positive. If sex linkage were involved, no positive daughters should occur in this series, and 50 per cent of the sons should be positive. The possibility of sex linkage is, therefore, excluded. These data are fully consistent with the conclusion that the hereditary mechanism involved is a single autosomal recessive gene.

In order to test the universality of these results, analyses were made of three additional sets of data, as follows: 273 families of the children in attendance at the Pediatric Cardiac Clinic (the family incidence based on histories); 129 family trees of negative parents published in the British Medical Research Council Report (3); and 50 family trees collected by Draper (7).

In each of the three sets of data the incidence among siblings of negative parents was lower than the incidence where one parent was positive. When both parents were positive the family incidence was still higher. In Table XII is presented

TABLE XII

*Comparison of observed incidence with expectation from Mendelian formulae\**

Name of hospital	Number of siblings in each family	Number of families	Observed number	Expected number	Difference	$\sigma^2$
St. Thomas' Hospital	1	10	10	10		
	2	19	23	21.7	1.3	2.33
	3	19	21	24.6	3.6	5.00
	4	9	11	12.2	1.2	3.78
	5	12	17	19.7	2.7	7.10
	6	5	9	11.0	2.0	3.88
	7	1	2	2.0	0	0.97
	8	2	5	4.4	0.6	2.35
	9	2	3	4.9	1.9	2.78
	Total	79	101	110.5		28.19
					1.8 $\sigma$	=5.3
Children's Hospital	1	2	2	2		
	2	5	5	5.7	0.7	0.61
	3	7	7	9.1	2.1	1.84
	4	5	9	7.3	1.7	2.10
	5	1	1	1.6	0.6	0.59
	6	3	4	5.5	1.5	2.33
	7	1	1	2.0	1.0	0.97
	8	2	5	4.4	0.6	2.35
	9	2	6	4.9	1.1	2.78
	10	1	2	2.9	0.9	1.81
	Total	29	42	43.4		15.38
					0.4 $\sigma$	=3.9

\* Analysis of pedigrees published in the British Medical Research Council (10).

a detailed analysis of the data derived from the two London hospitals. These data are quite consistent with those cited on the 122 families investigated in detail.

### Comment

The suggestion that an hereditary susceptibility underlies the *familial* incidence of rheumatic fever, is not, of course, new. A study of the literature shows that several attempts have been made to examine the *familial* incidence of rheumatism in genetic terms. The results, however, have in no instance been conclusive, chiefly because satisfactory genetic methods had not been applied to the data.

Some workers have based their views on a study of family pedigrees. The difficulty with this approach is that most matings in any pedigree are usually of no critical importance for analytical purposes. Even an extensive pedigree does not obviate the necessity of special study of the critical cases.

Another practice has been to add all families together, regardless of parentage. The results of such computations cannot be interpreted significantly, inasmuch as the expectations are so different in families of negative parents as compared with families with one or both parents positive, that an estimate of the agreement between genetic theory and observations cannot be made.

A third practice among investigators interested in the hereditary aspects of the disease consists in adding together all cases, regardless of the size of the families, and treating the total as a unit for comparison. This procedure is not permissible for studies on rheumatic fever because of the way in which the data are collected. On any genetic basis the frequency of rheumatic children should be smaller for the offspring of negative parents than it is for progeny having one or both parents positive. However, much, if not all, of the data on the *familial* incidence is gathered from among the children in attendance at a cardiac clinic. Consequently, the families used in such a study will necessarily have at least one rheumatic sibling. All summaries will, therefore, show that all families of one sibling only will be rheumatic, regardless of the presence or absence of the disease in the parents. This source of error is also present in families of larger size, but its magnitude de-

creases rapidly as the size of the family increases. Such a selection does not necessarily vitiate the results because it is possible to introduce a simple correction for these omitted cases (15). It must be done separately, however, for each size of family.

Our data, which were also selected from a children's cardiac clinic, were therefore corrected for this factor. Lack of recognition of this source of error may account for the failure of some previous investigators to arrive at definite conclusions from their genetic studies. Much of the published data on this disease were not compared with our results because the summaries of the data could not be separated into components of parental diagnosis or family size or both. Selection of families from members of an adult rheumatic clinic would not need this correction for size of family. An attempt to make such a study proved futile since the progeny of such families was invariably small.

The possibility of inadvertent bias in the selection of the original sample so as to favor the interpretation of genetic transmission has to be considered. It would be quite possible that unknown selective factors might have been operative in any small sample, and perhaps even in the average of a larger series. It would be expected, however, that any large number of individual family groups would show widely different values for the deviation from theory if selective factors were involved. In Table IX, in the last column, the deviation from theory is given for each of 23 samples of parental type and family size (not including families of one sibling only). In no case is it as large as 2, and in only three cases does it exceed 1.3. It is evident then that this factor is not operative in the 122 families studied.

The demonstration of the mechanism of heredity in rheumatic fever makes it possible to prepare tables of predictions of the probable occurrence of the disease in the progeny of a given mating (with the reservation already stated regarding the limitations of our analysis to families from the lower income groups for New York City environment). Table XIII includes a sample of the types of matings that occur with some frequency. Here it may be seen that if one child has the disease, both parents transmit it, whether



TABLE XIII

*Table of prediction for rheumatic siblings in families of certain matings*

Mate A	Mate B	Family A pedigree	Family B pedigree	Predictions	
				All siblings	After 1+ occurs
+	+	+ or -	+ or -	100+	100+
+	-	+ or -	1 parent +	50+	50+
+	-	+ or -	1 sibling +	33+	50+
+	-	+ or -	Aunt or uncle +	8+	50+
+	-	+ or -	Most aunts or uncles +	25+	50+
+	-	+ or -	-	<5+	50+
-	-	-	1 parent +	<5+	25+
-	-	-	1 sibling +	<5+	25+
-	-	-	Aunt or uncle +	<5+	25+
-	-	-	-	<5+	25+
-	-	1 parent +	1 parent +	25+	25+

< less than.

they show it or not, and that the subsequent incidence in the family will be 25 per cent, 50 per cent, or 100 per cent, depending on whether neither, one, or both parents have rheumatic fever. If prediction is to be made before the birth of any sibling, the incidence of rheumatic fever in the population must be considered. We have assumed this figure to be less than 10 per cent. A more detailed consideration of the genetic phases of this investigation is now in preparation.

#### GENERAL DISCUSSION

We are cognizant of certain possible inaccuracies inherent in the material which has been subject to analysis. Although these families have been under close and exact observation for a period of years, the nature of the disease itself has made the problem a difficult one. Our incomplete knowledge of the etiology and epidemiology of rheumatic fever has required us to make certain assumptions which may prove to be unwarranted. However, on the basis of our present knowledge, accepted diagnostic criteria, and modern genetic methods of analysis, the observations presented will be discussed.

One important contribution of genetic research to clinical medicine is that it admits of numerical predictions in a given situation so that when alternative hypotheses are being considered, it is possible to make a choice based on the agreement

between an observed value and those which would be predicted from the several theories. On the basis of a postulated hereditary mechanism, predictions for progeny of different types of matings may be made.

It would seem fair to conclude from the genetic analysis of our data that the susceptibility for rheumatic fever is transmitted as a single autosomal recessive gene. This may be said with some assurance, since it is based on quantitative agreement between observed incidence and the value predicted from this hypothesis.

A consideration of penetrance<sup>4</sup> is of importance in diseases where hereditary factors play a rôle. Penetrance may be defined as the percentage of hereditarily susceptible individuals which manifests a trait (in this case, rheumatic fever). In general genetics, as well as in the study of many human traits, it is common to find that not all individuals with similar genetic backgrounds show the character or show it fully. When every genetically similar individual expresses the trait, penetrance is said to be 100 per cent. On the other hand, if the penetrance is very low, approaching 0 per cent, it may be very difficult to establish the hereditary mechanism.

In the case of a recessive disease, the best method of estimating penetrance is from the progeny of positive parents. In our series, four families where both parents were positive had 15 siblings. Of these, 13 were rheumatic. Of the remaining two individuals, one is now seven years old and the other is fifteen. The penetrance is, therefore, 86 per cent. We have examined from our general clinic 13 additional siblings in 5 similar matings, finding 10 rheumatic; taking all the cases together gives a penetrance of 82 per cent.

The occurrence of even a single exception in the progeny of two positive parents has sometimes been advanced as evidence against the interpretation of recessiveness. From the foregoing discussion, it is apparent that this would be true if penetrance were found to be 100 per cent in the progeny of other types of matings. Our negative cases, therefore, cannot be regarded as unfavorable to the interpretation we have made.

<sup>4</sup> We wish to acknowledge our indebtedness to Dr. H. J. Muller for helpful suggestions in the discussion of penetrance.



The failure of a dominant factor to be expressed in any generation may be attributed to poor penetrance. The high penetrance observed in families of positive parents in these families excludes this possibility for our cases.

From the clinical viewpoint the penetrance of rheumatic fever has greater interest than any other single feature of its genetic pattern. If the penetrance is found to be lower in different geographical localities or economic groups, or following particular changes in the environment, important progress will be possible in the prevention of this disease.

In clinical investigation it would be advisable to select, not a sample of the general population, but a series of subjects whose genetic constitutions are known. The evaluation of the efficacy of proposed treatment would be the ratio of positives in the series compared with the predicted incidence for their known genetic background.

If the incidence of rheumatic fever varies in different geographical localities and is lower among families of the well-to-do, it would not be expected that the mechanism of the hereditary susceptibility would be different but that variation in penetrance is the responsible factor.

A correction for variable penetrance has not been introduced in the table of predictions formulated for rheumatic siblings. Its effect would be to lower slightly the prediction for each mating. Since the actual incidence of rheumatic fever in the general population is not known, the predictions given would be correct only if the incidence is not more than 10 per cent.

Accepting the hereditary transmission of a susceptibility to rheumatic fever, it is interesting to consider whether every susceptible individual will necessarily develop rheumatic fever, or whether the development of the disease is dependent on other factors. By analogy with other human hereditary diseases, either hypothesis is possible. From what we know of the disease, the latter is more likely, namely, that other contributing factors are involved, such as environment and exposure.

The influence of environment in its relation to the occurrence of rheumatic fever in susceptible individuals, cannot be completely discussed from our studies. Selection from a clinic obviously

excluded rheumatic families of the well-to-do where the disease is relatively infrequent. Families living in localities in which the incidence of the disease is believed to be low, are not represented. It is, however, evident from our observations that in these rheumatic families living in New York City, unfavorable environmental conditions were not the determining factor influencing the *familial* incidence of the disease. The incidence of rheumatic fever in one-third of the families living under relatively favorable environmental conditions was comparable to that observed in two-thirds of the families where the environmental conditions were more unfavorable. (These observations are in accord with the report of the British Medical Research Council (4).)

Should comparable studies of the *familial* incidence of rheumatic fever in families of the well-to-do, living under more favorable climatic conditions, reveal a diminished incidence of the disease among susceptible individuals of the family, a preventive therapeutic method would be available in this disease.

Our observations cannot be said to exclude the factor of contagion. The data secured, however, is not consistent with the expectation of most contagious diseases.

In the study of the spread of tuberculosis in families, Opie (17) found that in families where there are open cases of tuberculosis, contact resulted in twice the number of positive cases as did exposure to latent or negative sputum cases, and the incidence for extra-familial exposure was far less than either.

A comparable analysis of the spread of rheumatic fever in our families reveals that, unlike tuberculosis, no direct relation can be demonstrated between type and source of exposure and resulting disease. The analogy, however, is not strictly comparable since it is based on the assumption that the etiological agent was present in the nasopharynx of rheumatic individuals during activity and possibly carried during quiescence. It is conceivable that the virus may be present in the respiratory passages for only a short time, if at all. Had we been able to culture a virus from these individuals, different results might have been obtained.

Many observers believe that rheumatic fever may be spread only during a 'respiratory infection preceding rheumatic activity. The comparable incidence of rheumatic activity following "active" and "inactive" exposure, would not support this view. It is reasonable to suppose that the acquisition of rheumatic fever may be dependent on the exposure of the susceptible individual to an etiological agent that is widespread in this geographical environment.

In recent years the view that rheumatic fever is an allergic or immunological response of *susceptible* individuals to specific or nonspecific agents, has been stressed. The evidence presented of an hereditary susceptibility to rheumatic fever would seem to favor this hypothesis. It must, however, be stated that conclusive evidence in support of this concept has not yet been presented.

#### SUMMARY

1. There is presented a consideration of the rôle of environment, contagion and heredity as factors responsible for the familial incidence of rheumatic fever in 112 families, observed over a period ranging from 3 to 18 years.

2. There did not appear to be a direct relation between the environments studied and the incidence of rheumatic fever. One-third of the 112 families lived under relatively favorable environmental conditions, and two-thirds lived under unfavorable environmental conditions. In the former group the incidence of rheumatic siblings was 53 per cent, as compared with 46 per cent in the latter group.

3. There was no direct relation between the type and source of exposure and the resulting activity. The incidence of rheumatic fever following "active exposure," and "inactive exposure" was comparable. Intimate contact ("familial exposure") and casual contact ("extra-familial exposure") were equally effective.

(a) Only 21 per cent of 968 person years of "active exposure" could be related to subsequent rheumatic activity.

(b) In a total of 55 families with rheumatic parents, in 47 per cent, activity followed "active exposure"; in 53 per cent, activity followed "inactive exposure."

(c) In 57 families with non-rheumatic parents,

in 57 per cent, activity followed "extra-familial exposure" (casual contact); in 43 per cent, activity followed "familial exposure" (intimate contact).

(d) The interval between "active" or "inactive" exposure and the onset of rheumatism was 1 year in 20 per cent; 2 to 5 years in 49 per cent; and 6 to 11 years in 31 per cent.

4. The 227 rheumatic siblings experienced 588 calendar years of rheumatic activity. Of these years, 159, or 27 per cent, were simultaneous years of rheumatic activity.

(a) The interval between the related manifestations was: 1 month, 29 per cent; 1 to 2 months, 24 per cent; 2 to 11 months, 47 per cent. Three-fourths of the related manifestations of rheumatic activity were between joint pains and other rheumatic manifestations. One-fourth of the related manifestations were between polyarthritis, carditis and chorea. In 66 per cent the interval between these major manifestations was 2 to 11 months.

5. In 51 families, 59 parents (mother or father) were rheumatic; 44 per cent experienced rheumatic activity during the lifetime of the siblings. In no instance did a negative parent acquire the disease. The incidence of rheumatic siblings was comparable in families with a rheumatic mother or father.

6. Of 112 rheumatic families, 49 per cent had parental rheumatism. In only 28 per cent of the families were parents and pedigree on maternal and paternal sides apparently negative.

(a) Of a total of 468 siblings over the age of 3 years, 48 per cent were rheumatic; 46 per cent males and 54 per cent females.

7. All identical twins cited (4 pairs) were alike in having rheumatic fever. Of the 12 pairs of fraternal twins, 5 pairs had similar incidence, i.e., both positive or both negative, and 7 pairs had dissimilar incidence.

8. A genetic analysis of the data corrected for size of family gave agreement between observed and expected values.

(a) For children of 58 pairs of negative parents, the observed incidence was 94, the expected value 88.

(b) For children of 37 positive mothers, the observed incidence was 90, the expected value 86.

(c) For children of 29 positive fathers, the observed and expected incidence were respectively 29 and 27.9.

(d) The hereditary mechanism involved was a single autosomal recessive gene. Dominance, involving one or more genes, and recessives involving two or more genes, as well as sex linkage were all excluded.

#### CONCLUSION

1. These studies indicate that there is an hereditary factor distributed among the population which makes the bearer susceptible to rheumatic fever. This factor is transmitted as a single autosomal recessive gene.

2. The exact rôle of environment and contagion in the acquisition of the disease by susceptible individuals cannot be determined from these studies.

3. Hereditary susceptibility would seem to determine the *familial* incidence of rheumatic fever, but may not necessarily be the sole condition essential for the development of the disease.

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# SPLENIC VEIN PRESSURE IN CONGESTIVE SPLENOMEGALY (BANTI'S SYNDROME)

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The possibility that Banti's syndrome may be the result of some common factor operating within the portal system is suggested by the variety of disturbances that appear to be associated with this clinical picture (1, 2, 3, 4, 5, 6).

From a study of a relatively large number of patients presenting this syndrome of anemia, leukopenia and thrombocytopenia with splenomegaly, we were impressed by the possibility that a common factor might be increased portal vein pressure. We observed the Banti syndrome in association with cirrhosis of the liver, with partial and progressive obstruction of the splenic vein by neoplasms and thrombi, and in the later stages of schistosomiasis infestation. Portal hypertension might conceivably be present in all and portal hypertension might be a factor common to all.

If portal hypertension is a factor it should be possible to determine its presence and to measure its degree by direct readings at operation. Observations on the splenic vein pressure have now been made in 15 cases by inserting the needle of the venous pressure apparatus into the splenic vein after delivery of the spleen and before ligation of any of the larger splenic vessels.

It is important to have the level of the venous pressure manometer adjusted to the cardiac level before the operation is started. It is also important to be sure that the readings are taken without excessive traction on or distortion of the splenic vessels.

In several of the 15 cases studied technical surgical difficulties interfered, and the results are open to question. In two instances the exact clinical diagnosis remained doubtful even after operation and microscopic study of the liver and spleen.

There have been, however, 8 cases of Banti's syndrome in which the associated lesion could be definitely established and in which accurate venous pressure readings were obtained. In addition

there have been 3 cases of hemolytic jaundice to serve at least as a beginning of a control series.

Of the 8 cases with Banti's syndrome in 3, the splenomegaly was associated with chronic infestation by schistosoma *Mansoni*. In the schistosomiasis cases Banti's syndrome appeared gradually with slowly progressive splenic enlargement and slowly progressive anemia, leukopenia and thrombocytopenia. In all, sections of the removed spleen showed the diffuse fibrosis and the perifollicular congestion that is typical of the Banti type of splenomegaly, and liver biopsy revealed the typical schistosomal type of liver cirrhosis with remnants of ova in the periportal spaces. In all, splenectomy was followed by a prompt return of the blood values to normal.

TABLE I  
*Splenic vein pressure in 3 cases of Banti's syndrome associated with chronic schistosomiasis*

Case	Splenic vein pressure	Simultaneous arm vein pressure
	mm. H <sub>2</sub> O	mm. H <sub>2</sub> O
1. P. R.....	250	50
2. A. E.....	335	105
3. G. P.....	500	70

Splenic vein pressure readings were obtained in 5 cases of Banti's syndrome associated with the Laennec type of liver cirrhosis.

TABLE II  
*Splenic vein pressure in 5 cases of Banti's syndrome associated with Laennec's cirrhosis*

Case	Splenic vein pressure	Simultaneous arm venous pressure
	mm. H <sub>2</sub> O	mm. H <sub>2</sub> O
4. C. M.....	275	12
5. G. M.....	325	85
6. D. P.....	450	125
7. L. DeR.....	275	105
8. N. A.....	470	140

Three cases of typical hemolytic jaundice serve as the only controls we have as yet been able to study.

TABLE III

*Splenic vein pressure in 3 cases of typical hemolytic jaundice*

Case	Splenic vein pressure	Simultaneous arm venous pressure
	mm. H <sub>2</sub> O	mm. H <sub>2</sub> O
9. R. B.....	105	80
10. N. B.....	125	130
11. W. U.....	120	85

## DISCUSSION

The relatively great increase in splenic vein pressure in cases presenting Banti's syndrome when compared with the venous pressure simultaneously recorded in the arm suggests that portal hypertension may be an important factor in the production of the chronic splenomegaly.

The use of cases of hemolytic jaundice as controls is, of course, not entirely satisfactory, but the fact that in 3 instances the splenic and arm vein pressure were approximately the same suggests that portal hypertension is not a factor in this disease.

The importance of obtaining pressure readings in patients with cirrhosis but without splenomegaly is appreciated, and data on patients with chronic passive congestion due to cardiac failure would be of interest. The difficulties involved in obtaining such measurements are obvious.

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# STUDIES OF THE PRINCIPLE IN LIVER EFFECTIVE IN PERNICIOUS ANEMIA. IV. THE THERAPEUTIC ACTIVITY OF ITS MULTIPLE FACTORS<sup>1,2</sup>

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Early in the course of an investigation of the principle in liver effective in pernicious anemia certain observations suggested the possibility that the therapeutic activity of liver extract might depend upon the presence of several chemically distinct substances. The evidence for this view was the fact that continued purification of therapeutically active liver extract, in the absence of significant losses and of destructive procedures, resulted in partial or complete extinction of therapeutic activity. On the other hand, the admixture with such highly purified materials of other fractions derived from liver extract resulted in the recovery of therapeutic activity. These latter fractions we have termed *accessory* factors, for they appeared to augment the activity of the *primary* factor (or factors), while in the absence of the primary factor they were therapeutically inert. This communication describes in detail the observations mentioned above, part of which have already been presented in a preliminary report (1).

## METHODS AND MATERIALS

The patients studied were suffering from classical Addisonian pernicious anemia in relapse. Complications such as severe combined system disease and hemorrhage were absent. No infections occurred during the periods of observations, except for cystitis in three patients (Cases 7, 9, and 12, Table I). During the prolonged periods

of study the patients' diet was of a mixed type, including meat or fish once daily, but devoid of yeast, liver, kidney, and tripe. Prior to the administration of therapeutically active fractions the blood level of each patient was established by adequate control periods, as noted in Table I.

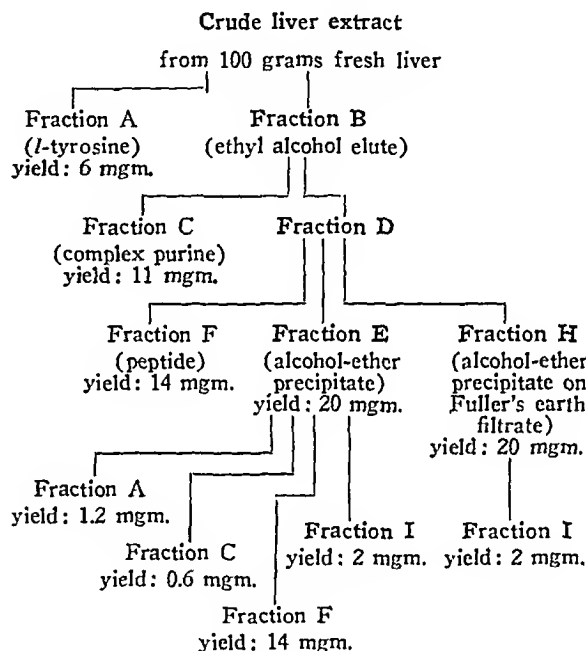


FIG. 1. DERIVATION OF PURIFIED LIVER FRACTIONS

The percentage of reticulocytes in the capillary blood was estimated by the wet method, 1000 erythrocytes being counted. Erythrocyte, hemoglobin, and hematocrit determinations were made on venous blood drawn without stasis, and rendered incoagulable by heparin. The number of erythrocytes was determined with pipettes and counting-chambers certified by the Bureau of Standards. The volume of packed red blood cells was measured in the Wintrobe hematocrit. The hemoglobin was determined by the Stadie-Wu method (12). The reticulocytes were counted

<sup>1</sup> Presented in part at the meeting of the American Society for Clinical Investigation, Atlantic City, May 6, 1935.

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TABLE I  
Data of Figure 2

Curve	Patient	Length of period	Date of period	Fractions administered					Calculated amount of primary fraction administered per day	Rate of administration	Control period preceding administration
				E	H	A	C	F			
		days		Total number grams fresh liver from which extract derived					grams fresh liver from which extract derived		days
1	1	20	Dec. 18, 1935 to Jan. 7, 1936	200		100	20		10	Every 2d day	1
2	2	30	April 16 to May 16, 1936		900	0	0	900	30	Every 10th day	2
3	3	28	April 17 to May 15, 1936		300	300	300	0	11	Every 10th day	16†
4	3	15	April 2 to April 17, 1936		370	0	0	0	25	Single	1
5	4	30	Dec. 28, 1935 to Jan. 27, 1936	300		110	200		10	Every 2d day	10†
6	4	9	Dec. 19 to Dec. 28, 1935	100		0	0		11	Every 2d day	1
7	5	14	Mar. 20 to April 3, 1936		100	0	0	100	7	Single	1
8	1	20	Feb. 5 to Feb. 25, 1936	200		*	200		10	Every 2d day	50†
9	6	18	Dec. 9 to Dec. 27, 1935	240		0	0		13	Every 2d day	10
10	7	8	April 30 to May 8, 1936		100	100	100	100	13	Single	7
11	4	10	Jan. 27 to Feb. 6, 1936	100		100	100		10	Every 2d day	40†
12	8	24	Sept. 27 to Oct. 21, 1935	240		240	240		10	Every 2d day	3
13	9	8	May 1 to May 9, 1936		100	100	100	100	13	Single	11
14	1	20	Jan. 7 to Jan. 27, 1936	200		300	200		10	Every 2d day	21†
15	10	10	June 5 to June 15, 1936		100	100	100	100	10	Single	75
16	11	21	Oct. 11 to Nov. 1, 1935	200		200	200		10	Every 10th day	6
17	12	41	Oct. 2 to Nov. 12, 1936		600	600	600	600	15	Every 2d day during first 20 days, every 10th day thereafter	29
18	13	14	Oct. 25 to Nov. 8, 1935	100		100	100		7	Single	22
19	2	27	May 16 to June 12, 1936		300	300	300	300	11	Every 10th day	32†
20	1	16	Feb. 25 to Mar. 12, 1936	100		100	100		6	Every 2d day during first 8 days	70†
21	1	16	Mar. 23 to April 8, 1936	100		†	100		6	Every 2d day during first 8 days	97†
22	6	12	Dec. 27, 1935 to Jan. 8, 1936	170		170	170		14	Every 2d day	28†

\* 100 mgm. Fraction A orally daily.

† 1.0 gram Fraction A orally daily.

‡ Includes preceding experimental period.

daily and the venous blood constituents were studied, in most cases, on alternate days.

The derivation from commercial liver extract (Fraction G of Cohn et al. (2)) of the materials discussed in this communication is presented in Figure 1. In this figure the fractions containing the primary factor are depicted by heavy type. They are Fractions B, D, E, H, and I. The details of the preparation of Fractions A, B, and C have been previously described (3, 4). From Fraction D the primary factor has been brought down in both of the amorphous fractions E and H. From Fraction D, also, was separated Fraction F (an accessory factor) by precipitation with rhodanilic acid (5). The sources of primary factor discussed in this communication are Fraction E and Fraction H. While Fraction E contains both primary factor and Fraction F, as well as very small amounts of Fractions A and C, Fraction H, although amorphous, is completely devoid of Fractions A, C, or F. Fraction I was obtained from either Fraction E or from Fraction H, by precipitation with Reinecke salt and subsequent regeneration, as a microcrystalline sulfate in a yield of 2 mgm. from 100 grams of fresh liver (6). Studies of the therapeutic action of Fraction I will be presented in a later communication.

The accessory factors are represented by Fractions A, C, and F. Fraction A, obtained in a yield of 6 mgm. from 100 grams of liver, has been identified as *l*-tyrosine (3). In most of the experiments recorded below crystalline commercial *l*-tyrosine (Kahlbaum or Eastman Kodak) has been employed instead of Fraction A isolated from liver. Fraction C, obtained in a yield of 11 mgm. from 100 grams of liver, has been identified as a complex purine (3) and was administered in a crystalline state. Fraction F, a peptide, was prepared either directly from Fraction D or from Fraction E, in a yield of 14 mgm. from 100 grams of liver (5), and has been administered as a regenerated crystalline rhodanilate.

Throughout the experiments recorded below Fractions E (with the exception of the material administered to Patient 10, Table I) and H have been obtained from the same original supply of each fraction prepared in a large amount. The following quantities of the various fractions have been administered, as derived from 100 grams of

fresh liver: Fraction A, 11 mgm.; Fraction C, 6 mgm.; Fraction F, 14 mgm.; Fraction E, 20 mgm.; and Fraction H, 20 mgm.

Unless otherwise noted, all of the fractions were sterilized by boiling for one minute on the weakly acid side (alkaline to methyl red and acid to phenol red), and were administered by intramuscular injection.

*The therapeutic activity of the primary factor, with and without the accessory factors*

The therapeutic activity of the primary factor *without*<sup>3</sup> the three accessory factors, administered as either Fraction E or H, was studied in six patients during nine periods of observation. These fractions were administered during periods varying from nine to thirty days in length, in amounts derived from 7 to 30 grams of liver per day. The effects of these fractions upon erythrocyte production are depicted in the left-hand part of Figure 2. The primary factor as either Fraction E or H, *together with* the three accessory factors, was administered to eleven patients during thirteen periods of observation from eight to forty-one days in length, in amounts derived from 6 to 15 grams of liver per day. Included among these latter patients were four to whom had been previously administered primary factor without the accessory factors. In the right-hand part of Figure 2 are depicted the individual erythrocyte regeneration curves following the administration of the primary factor together with the three accessory factors. The fractions administered, the amounts, and other relevant data bearing upon these erythrocyte regeneration curves are noted in Table I.

It is evident from inspection of Figure 2 that, in the *absence* of the three accessory factors, the effect of either Fraction E or H upon erythrocyte production (with the exception of Curve 2; *vide infra*) was either slight and of short duration (Curves 1, 3, 4, 7, 9), or was entirely lacking (Curves 5, 6, 8). On the other hand, after the administration of the primary factor *together with* Fractions A, C, and F, the erythrocyte responses showed a high degree of activity.

<sup>3</sup> Although Fraction E contains Fraction F, and very small quantities of Fractions A and C (Figure 1), for the purpose of the present discussion it is considered in conjunction with Fraction H. Fraction H is completely devoid of Fractions A, C, and F.



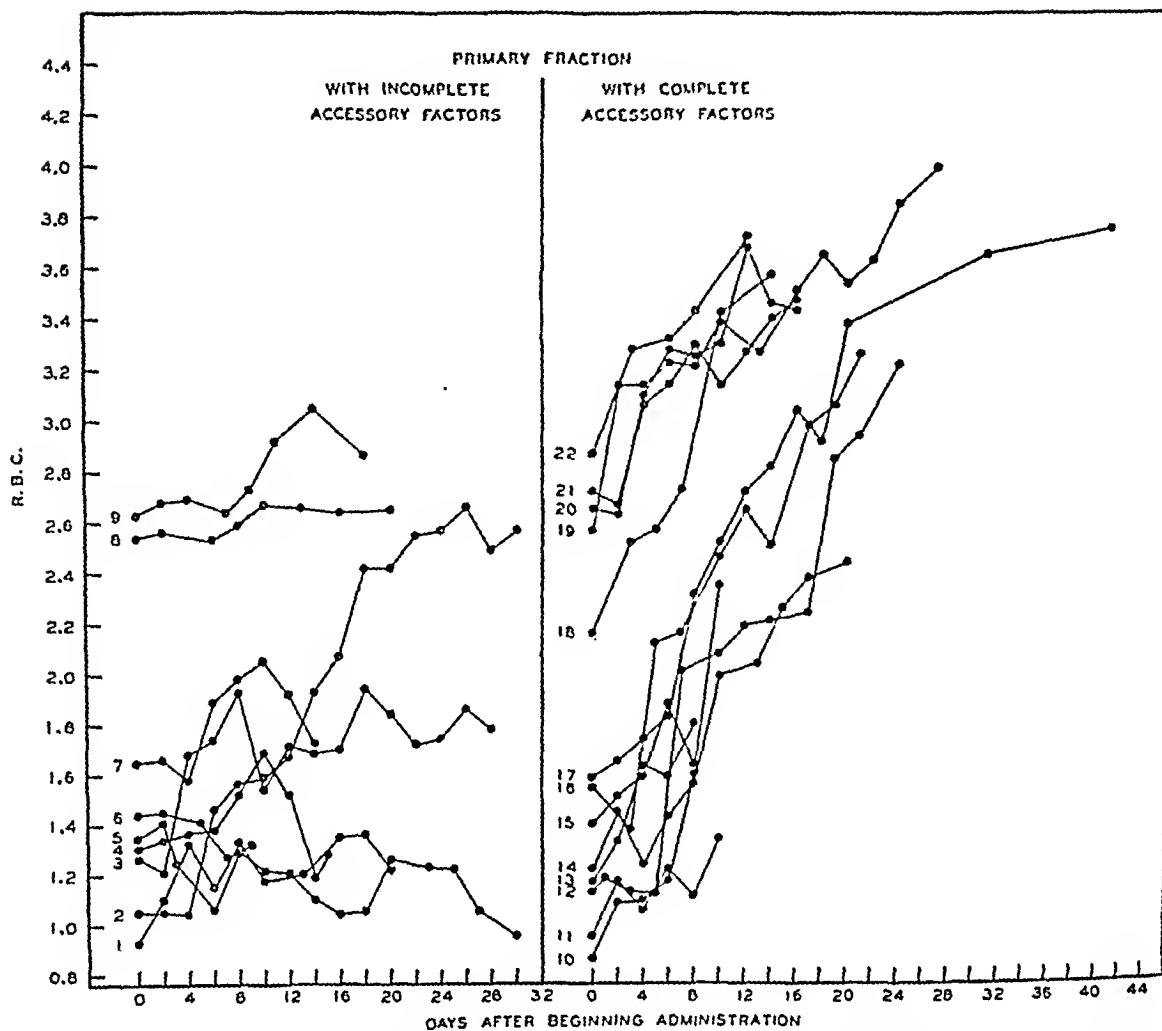


FIG. 2. ERYTHROCYTE REGENERATION CURVES FOLLOWING THE ADMINISTRATION OF PRIMARY FACTOR WITH INCOMPLETE AND WITH COMPLETE ACCESSORY FACTORS. FRACTIONS ADMINISTERED, QUANTITIES, AND OTHER DATA CONTAINED IN TABLE I

TABLE II

*Comparative reticulocyte responses to primary factor with and without accessory factors*

Fraction.....	Primary factor with incomplete accessory factors						Primary factor with complete accessory factors			
	E	H	H	E	H	E	H	E	E	H
Patient.....	1	2	3	4	5	6	7	8	11	12
Curve number in Figure 2.....	1	2	4	5	7	9	10	12	16	17
Total amount fresh liver from which extract derived, grams.....	100	300	370	100	100	200	100	100	100	200
Rate of administration.....	-20 grams every 2d day*	Single dose	Single dose	-20 grams every 2d day	Single dose	-40 grams every 2d day	Single dose	-20 grams every 2d day	Single dose	-40 grams every 2d day
R.B.C. at beginning, millions per cu. mm.	0.93	1.05	1.32	1.44	1.65	2.62	0.86	1.15	1.56	1.57
R.B.C. at end, millions per cu. mm.....	1.19	1.58	1.69	1.32	2.05	2.73	1.60	2.08	2.46	2.52
Reticulocytes at peak, per cent.....	7.8	31.0	16.4	3.0	9.8	4.4	24.6	25.0	23.8	18.1
Predicted reticulocyte peak, per cent....	34	31	25	24	20	9	35	28	21	21
Day of reticulocyte peak.....	6th	6th	7th	6th	6th	8th	5th	7th	4th	8th
Length of period, days.....	10	10	10	9	10	9	8	10	9	10

\* The abbreviation -20 stands for "derived from 20 grams of liver."

All of the curves of Figure 2 are represented in the average erythrocyte regeneration curves of Figure 3. To facilitate interpretation the curves representing initial erythrocyte levels below 2.0 million have been averaged separately from those representing initial levels above 2.0 million. It is evident that the administration of the primary factor, together with Fractions A, C, and F, resulted in a rate of erythrocyte regeneration markedly greater than that produced by the primary factor in the absence of all three of the accessory factors, despite the administration, in the latter instance, of considerably larger quantities of pri-

mary factor. Indeed, the administration of both primary and accessory factors resulted in a rate of erythrocyte production closely approximating that induced by the intramuscular administration of an identical average daily dosage (derived from 11 grams of liver) of a commercial liver extract recently studied by Murphy (7).

Data concerning reticulocyte production following the administration of the primary factor *alone* were obtained in six patients, whose initial erythrocyte levels permitted a possible reticulocyte response. These observations are presented in Table II. For comparison there are included the

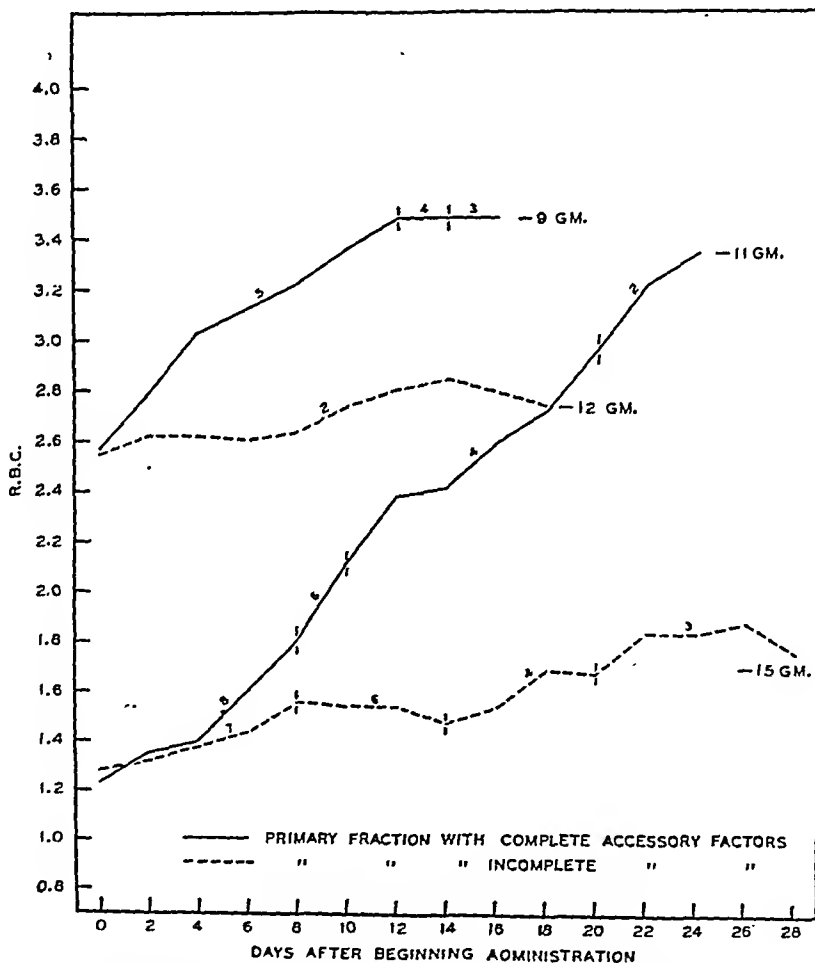


FIG. 3. AVERAGES OF ALL ERYTHROCYTE REGENERATION CURVES CONTAINED IN FIGURE 1, AT TWO DIFFERENT INITIAL ERYTHROCYTE LEVELS

The number of separate experiments included in each average is represented by the numerals directly above each curve; the periods corresponding to these numerals are defined by the small vertical lines. The quantity at the end of each curve denotes the calculated average daily amount, in terms of fresh liver from which the extract was derived, of primary fraction administered.

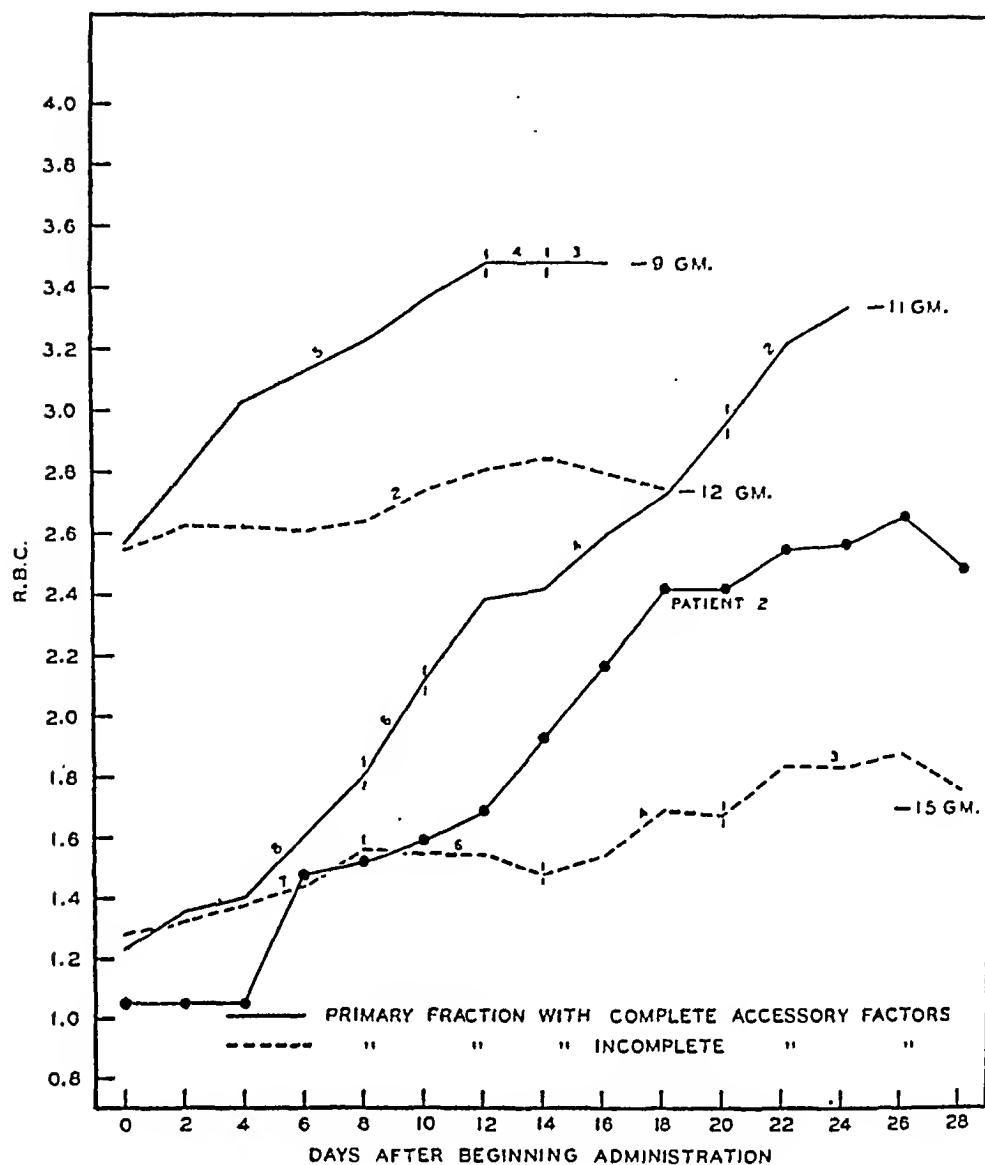


FIG. 4. ERYTHROCYTE RESPONSE TO ADMINISTRATION OF MASSIVE AMOUNT (—300 GRAMS EVERY TENTH DAY) OF PRIMARY FACTOR WITH INCOMPLETE ACCESSORY FACTORS (PATIENT 2)

This erythrocyte regeneration curve is superimposed on the curves of Figure 3, for comparison with average response to administration of smaller amount of primary factor together with complete accessory factors. See text.

predicted reticulocyte peaks, based upon unpublished data of the authors, following the intramuscular injection of commercial liver extract derived from 100 grams of liver. It is evident that Fraction E or Fraction H, when administered in an amount derived from 100 or 200 grams of liver, during the first ten days, induced markedly submaximal reticulocyte responses, regardless of the rate of administration (single dose in Patient 5, divided doses in Patients 1, 4, and 6). After the administration of a massive amount of Fraction H (derived from 300 grams of liver) a maximal response followed in Patient 2, but an even

larger amount (derived from 370 grams of liver) administered to Patient 3 was followed by a submaximal response. On the other hand, in four patients treated with moderate amounts of Fractions E or H, *together* with the three accessory factors, the reticulocyte responses were approximately maximal. (See Table II.)

*The effects of massive amounts of the primary factor with incomplete accessory factors*

The therapeutic effects of large amounts of the primary factor *alone* were studied during a prolonged period in one patient. The erythrocyte

regeneration curve of this case (Patient 2) is included in Figure 2 (Curve 2), and is also presented in Figure 4, in order to facilitate comparison with the average erythrocyte regeneration curves of Figure 2. To this patient (Figure 4) at an initial erythrocyte level of 1.05 million, were administered Fractions H and F, each derived from 300 grams of liver, in a single dose. The same amount of each fraction was again administered on the tenth and on the twentieth days, totaling material derived from 900 grams of liver during thirty days. The reticulocyte response was maximal, reaching a peak of 31.0 per cent on the sixth day. During the first ten days the erythrocytes rose to 1.58 million, by the thirtieth day they reached a level of 2.5 million. It is apparent, from the data of Figure 4, that the erythrocyte response to the administration of large amounts (—30 grams per day)<sup>4</sup> of the primary factor, without the three accessory factors, was not as great as the average erythrocyte response to smaller amounts (—11 grams per day) of primary factor together with the accessory factors. At the end of this period Fractions H, A, C, and F, each derived from 100 grams of liver, were administered to this patient, and the same dosage was repeated on the tenth and on the twentieth days. The erythrocytes continued to rise, reaching a count of 4.0 million on the twenty-seventh day (Curve 19, Figure 2).

#### *The augmentative action of each accessory factor*

In the studies described above all three of the accessory factors, Fractions A, C, and F, were administered together with the primary factor (Fraction E or H). That each of these fractions may act as an accessory factor was suggested by certain indirect evidence (1). Direct evidence for this view was furnished by the following observations.

The administration of an adequate amount of primary factor together with Fractions C and F, but with an incomplete amount of Fraction A, was followed, in two patients, by no erythrocyte response. To Patient 4 (Curve 5, Figure 2), at an initial erythrocyte level of 1.3 million, were administered on alternate days Fractions E — 20

grams (derived from 20 grams of fresh liver), C — 10 grams, and A — 2 grams, during the first ten days; during the second ten days the dosage on alternate days was E — 20 grams, C — 10 grams, and A — 10 grams; and during the last ten days, E — 20 grams, C — 20 grams, and A — 10 grams. It is seen that even after the increase of Fraction C, in the absence of a complete amount of Fraction A, during the last ten days, the erythrocytes continued to fall, reaching 0.95 million. To the patient were then administered on alternate days doses of Fractions E — 20 grams, C — 20 grams, and A — 20 grams, during the following ten days, and, as depicted in Curve 11, Figure 2, the erythrocytes rose to 1.34 million, accompanied by clinical improvement. Similar observations were made in Patient 1, at an initial erythrocyte level of 2.54 million. As shown in Figure 6, to this patient were administered on alternate days doses, during a period of twenty days, of Fractions E — 20 grams and C — 20 grams, as well as 100 mgm. of tyrosine (Fraction A), *orally*, daily. The erythrocytes remained stationary. In the next succeeding period the same amounts of Fractions E and C were continued, but Fraction A — 20 grams, was administered on alternate days by *intramuscular injection*. A satisfactory erythrocyte response promptly followed.

The accessory action of Fraction C, in the presence of an adequate amount of the other factors, was suggested by the following observations upon Patient 1, presented in Figure 5. During these observations over a period of forty days Fraction E — 20 grams was administered on alternate days. During the first twenty days Fraction C — 2 grams was also administered at the same rate. During the first ten days no additional Fraction A was injected (other than that contained in Fraction E; see Figure 1). It is seen that during this first period the reticulocytes rose to a peak of 9.2 per cent on the eighth day, followed by a rise of erythrocytes from 0.92 million to 1.2 million. Slight clinical improvement took place. During the second period of ten days, Fraction A — 20 grams, administered on alternate days, was added to the previous dosage of Fractions E and C. In response to these materials the reticulocytes did not continue to fall, but rose again to a peak of 9

<sup>4</sup> The abbreviation — 30 grams per day stands for "derived from 30 grams of liver per day."

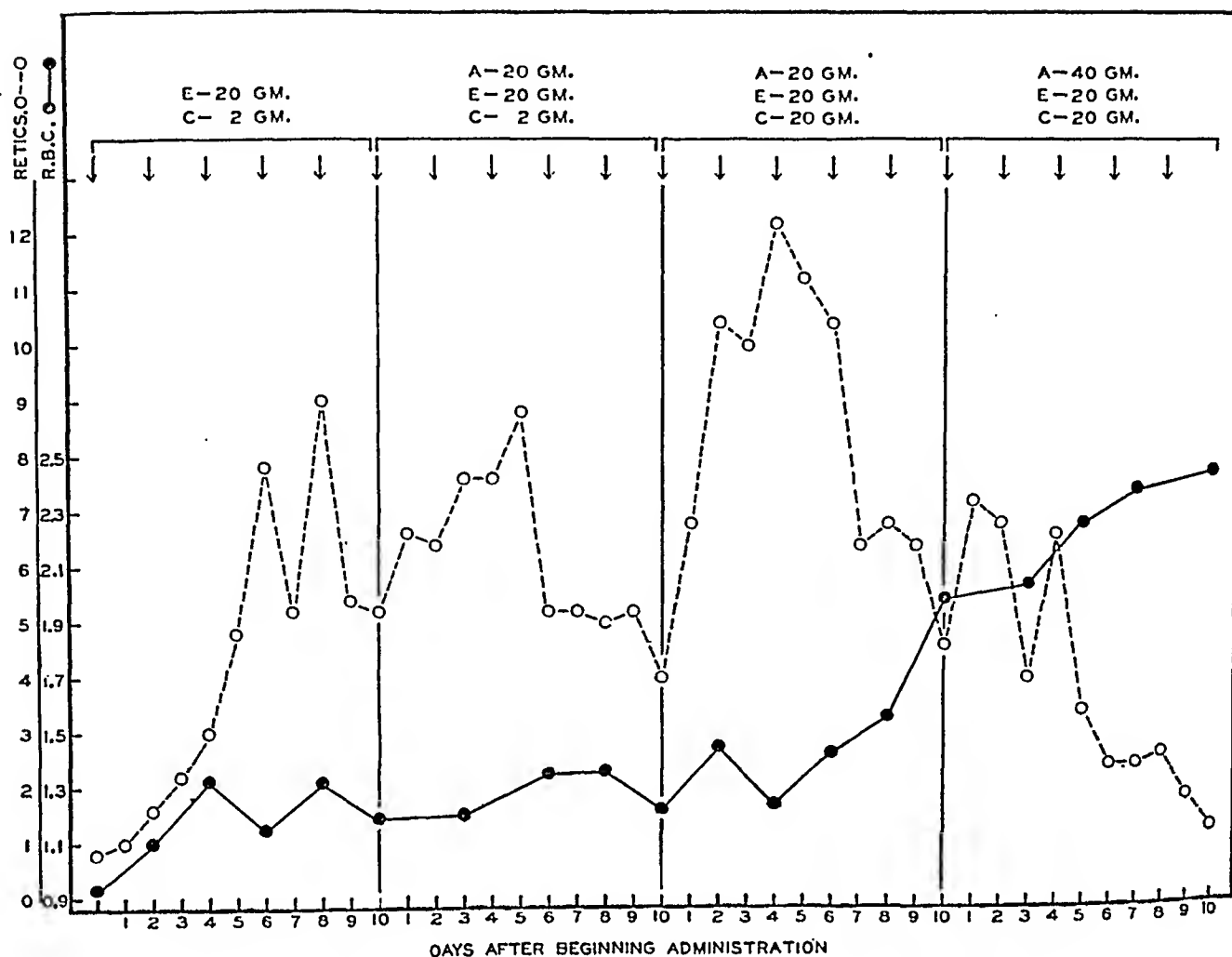


FIG. 5. PATIENT 1. FRACTION C AS AN ACCESSORY HEMATOPOIETIC FACTOR

The quantities above each period refer to the amounts of fractions injected on the days denoted by each arrow. Throughout the four periods Fraction E — 20 grams was administered on alternate days. During the first two periods Fraction C — 2 grams was administered on alternate days. There were only slight reticulocyte and erythrocyte responses in the first period in the absence of Fraction A and no further erythrocyte rise in the second period after addition of Fraction A — 20 grams on alternate days. The increase of Fraction C to — 20 grams in the third period resulted in the greatest rise of reticulocytes and satisfactory rise of erythrocytes, continuing in the fourth period.

per cent; the erythrocytes remained stationary, and the clinical condition was unchanged. During the third period the dosage of Fraction C on alternate days was increased from — 2 to — 20 grams, while the same amounts of Fractions E and A were continued. A third and distinctly orderly reticulocyte response, with a maximum of 12.4 per cent on the fourth day, was induced, and the erythrocytes rose from a level of 1.2 million to one of 2.0 million. Marked clinical improvement accompanied the changes in the blood. During the fourth period of ten days the continued administration on alternate days of the same fractions, except for an increase of the dosage of Fraction A from — 20 to — 40 grams, was ac-

companied by a continued rise of the erythrocytes. Thus the continuous administration of primary factor, as Fraction E, together with Fraction A, but with minimal amounts of Fraction C, induced only slight reticulocyte and erythrocyte responses. A ten-fold increase in the amount of Fraction C (third period, Figure 5), however, was followed by a reticulocyte response of greater magnitude, and by a satisfactory gain of erythrocytes. That Fractions E, A, and C, each derived from 20 grams of liver, were therapeutically more effective than Fractions E — 20, A — 20, and C — 2, is suggested in view of the discussion of double reticulocyte responses by Minot and Castle (8).

The accessory action of Fraction F, in the

presence of the primary factor together with Fractions A and C, was indicated by the following observations. To Patient 3 (Curve 4, Figure 2) at an initial erythrocyte level of 1.3 million was administered a single dose of Fraction H — 370 grams. (It is to be recalled that Fraction H, in contrast with Fraction E, is completely devoid of Fractions A, C, and F.) During the following fifteen days the erythrocytes rose slightly but then receded to a final value of 1.28 million. During the succeeding twenty-eight days (Curve 3, Figure 2), to Fraction H — 100 grams were added Fractions A — 100 grams and C — 100 grams. These amounts were administered every tenth day. On the seventh day the reticulocytes rose to a peak of 24.4 per cent, followed by a rise of the erythrocytes to 1.93 million on the eighth day. Thereafter, the erythrocytes rose no further, and by the twenty-eighth day had declined to 1.78 million. At this point commercial liver extract was administered, followed by rapid rise of the erythrocytes. Thus, in the complete absence of Fraction F, Fractions H, A, and C induced a rise of erythrocytes of only 0.5 million during a period of twenty-eight days. On the other hand, after the addition of Fraction F, Fractions H, A, and C, administered in similar dosage, induced satisfactory erythrocyte responses in five other patients (Curves 10, 13, 15, 17, and 19, Figure 2).

*The inactivity of the accessory factors in the absence of the primary factor*

In Table III are presented data concerning the negative therapeutic activity of the three accessory factors, Fractions A, C, and F, in the *absence* of the primary factor. After the administration of the stated amounts of accessory factors in a single dose observations of the reticulocytes and of the erythrocytes were made during periods ranging from seven to twenty days. In subsequent periods either commercial liver extract, or partially purified experimental liver extract, was administered, with resulting satisfactory responses in each case. It is seen that neither Fractions A, C, or F individually or together (Patients 10, 20, and 21, Table III) induced significant rises of reticulocytes or erythrocyte responses, in patients who subsequently reacted to the administration of crude liver extracts.

*Parenterally contrasted with orally administered tyrosine as an accessory factor*

The experimental evidence discussed above, that as little as 6 mgm. of *l*-tyrosine (Fraction A — 100 grams liver), when administered by intramuscular injection, acted as an accessory factor, is difficult to reconcile with the apparent fact that the patients who formed the subjects of this investigation derived several *grams* of tyrosine daily from ingested protein. The patients consumed a normal amount of protein, severe diarrhea was absent, no gross evidence of defective protein digestion was manifest, and during the experimental periods most of the patients gained in weight.

In the hope of throwing some light on this problem the following study was undertaken. Patient 1, following the observations recorded in Figure 5, remained without treatment for one week, during which the erythrocyte level remained stable. The experiment presented in Figure 6 was then instituted. During the first period of twenty days Fractions E — 20 grams and C — 20 grams were administered on alternate days. During this period also 100 mgm. of commercial *l*-tyrosine was administered *orally* every day. No change in the patient's clinical condition took place, and during the twenty days the erythrocytes rose from 2.55 million to only 2.65 million. During the first ten days of the following period the administration on alternate days of the same basic fractions, E — 20 grams and C — 20 grams, was continued, but only 1.2 mgm. of tyrosine (A — 20 grams) was *injected* intramuscularly on alternate days. However, a sharp rise of the erythrocytes to 3.48 million in sixteen days ensued, accompanied by clinical improvement.

During the nineteen days following the last treatment the erythrocytes declined to 2.72 million. At this point the same basic fractions E — 20 grams and C — 20 grams were administered, and continued on alternate days for ten days. During this period 1.0 gram of tyrosine was ingested daily. An erythrocyte response promptly followed, reaching in sixteen days 3.48 million. It is evident that the slope of the erythrocyte curve following the daily oral administration of 1.0 gram of tyrosine is very similar to that following the parenteral administration on alternate days of 1.2 mgm. of tyrosine.

TABLE III  
The inactivity of the accessory factors in the absence of the primary factor

Fraction.....	A		C			A + C		F	A + C + F		
	14	15	16	17	18	19	16	20	21	20	10
Patient.....											

	FIRST PERIODS Responses to administration of accessory factors										
	1.29	2.11	2.46	1.74	1.80	1.69	3.10	2.46	2.99	2.42	1.35
R.B.C. at beginning, millions per cu. mm.....											
R.B.C. at end, millions per cu. mm.....	1.29	2.12	2.47	1.51	1.75	1.51	2.90	2.42	3.03	2.51	1.40
Maximum reticulocyte count, per cent.....	3.4	3.8	1.2	4.0	3.4	2.0	2.4	1.4	3.0	1.2	4.6
Length of period, days.....	13	8	9	9	18	7	19	12	10	12	20
Date of period.....	Mar. 29 to April 11, 1934	April 19 to April 27, 1934	Jan. 3 to Jan. 12, 1935	Feb. 12 to Feb. 21, 1935	May 1 to May 19, 1936	Jan. 26 to Feb. 2, 1935	Feb. 6 to Feb. 25, 1935	April 26 to May 8, 1935	Jan. 13 to Jan. 23, 1936	May 8 to May 20, 1935	April 28 to May 18, 1936
Amount fresh liver from which extract derived, grams.....	137	150	100	200	500	90	150	900	100	125	300

	SECOND PERIODS Responses to administration of therapeutically active materials										
	C.l.e.*	E.l.e.†	E.l.e.	C.l.e.	E.l.e.	E.l.e.	E.l.e.	E.l.e.	E.l.e.	E.l.e.	E.l.e.
Material administered.....											
R.B.C. at beginning, millions per cu. mm.....	1.29	2.12	2.47	1.51	2.08	1.51	2.90	2.54	3.03	2.54	1.40
R.B.C. at end, millions per cu. mm.....	2.07	2.46	2.82	3.07	3.03	2.28	3.26	3.12	3.50	3.12	2.35
Reticulocyte peak, per cent..	31.9	9.6	7.8	26.4		26.6	6.6	6.8	3.2	6.8	12.0
Length of period, days.....	12	9	9	8	10	9	9	10	10	10	10
Date of period.....	April 11 to April 23, 1934	April 27 to May 6, 1934	Jan. 12 to Jan. 21, 1935	Feb. 21 to Mar. 1, 1935	June 10 to June 20, 1936	Feb. 2 to Feb. 11, 1935	Feb. 25 to Mar. 6, 1935	June 10 to June 20, 1935	Jan. 23 to Feb. 2, 1936	June 10 to June 20, 1935	May 18 to May 28, 1936
Amount fresh liver from which extract derived, grams.....	100	77	88	2000	100	200	150	100	100	100	200

\* Commercial liver extract.

† Experimental liver extract.

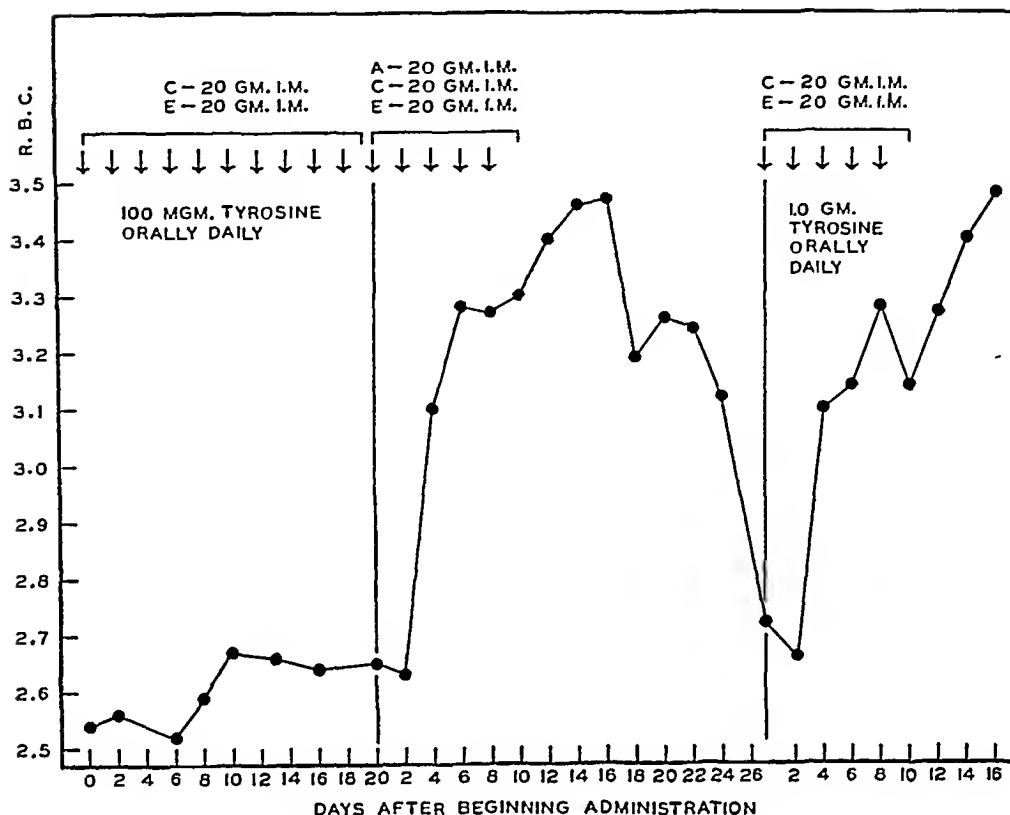


FIG. 6. PATIENT 1. PARENTERALLY CONTRASTED WITH ORALLY ADMINISTERED TYROSINE (FRACTION A) AS AN ACCESSORY HEMATOPOIETIC FACTOR

The quantities above each period refer to the amounts of fractions injected on the days denoted by each arrow. Fractions E and C were injected at a uniform rate in each of three periods. There was no erythrocyte response during the first period of twenty days while 100 mgm. of tyrosine was administered orally daily, followed by satisfactory rise of erythrocytes in the second period after 1.2 mgm. tyrosine was injected on alternate days. Following cessation of treatment, erythrocytes declined to previous level. In the third period, there was another satisfactory erythrocyte response after daily oral administration of 1.0 gram tyrosine.

It is thus suggested by these data that in this particular patient, at least, 100 mgm. of tyrosine ingested daily in addition to the tyrosine derived from the diet, was not equivalent in accessory hematopoietic activity to 0.6 mgm. (per day) of parenterally administered tyrosine, while a tenfold increase of the orally administered tyrosine was as active as the injected tyrosine.

#### DISCUSSION

The observations described above suggest that the complete therapeutic action of relatively crude liver extract in pernicious anemia was induced only by the presence in the extract of several chemically distinct substances. The evidence for

this hypothesis has been derived partly from data concerning reticulocyte production, but principally from data concerning erythrocyte regeneration during prolonged periods. One fraction has been termed a *primary* factor, the other fractions *accessory* factors. The primary factor alone, when administered in an amorphous impure state (Fractions E or H), exerted at most only a moderate therapeutic effect. The accessory factors (Fractions A, C, and F) were individually and collectively completely inert. On the other hand, the administration of the primary factor together with the three accessory factors was followed by satisfactory clinical improvement and by a rapid rate of erythrocyte regeneration.



It might be assumed that the satisfactory therapeutic activity of the four fractions together depended upon additive effects of a substance common to all. That this is unlikely is evidenced by the data of Table III. Fractions A, C, and F were individually and collectively administered in amounts sufficiently great, so that had there been contamination with the primary factor a response would have occurred. All of the evidence, rather, suggests that Fractions A, C, and F *augmented* the activity of a primary factor. Furthermore, it might be assumed that the entire therapeutic activity of liver extract resides in a single substance, and that the inadequate effects of our primary factor were due to the administration of insufficient amounts. Against this interpretation is the fact that even after the administration of an amount of primary factor derived from as much as 30 grams of liver per day (Curves 2 and 4, Figure 2), instead of the usual dosage of extract derived from 10 grams of liver per day, the resulting clinical and hematopoietic effects were inferior to those that followed the administration of smaller amounts (— 10 grams) of primary factor, *together with* like amounts of the three accessory factors.

We do not possess evidence that all possible accessory factors in crude liver extract are represented by Fractions A, C, and F. Nor do the present experimental data permit the conclusion that the four factors described are sufficient to induce a *complete* clinical and hematopoietic remission, and to maintain such a remission over a prolonged period. The longest periods over which continued observations have been made are the following. Patient 2, treated with Fractions H, A, C, and F, reached an erythrocyte level of 4.0 million in 27 days (Curve 19, Figure 2). Patient 12, whose course is depicted in Figure 2 (Curve 17), was treated with Fractions H, A, C, and F; at the end of 60 days the erythrocytes numbered 4.46 million.

Observations in one patient presented above have indicated that the accessory hematopoietic action of *l*-tyrosine (Fraction A), parenterally administered in an amount no greater than 0.6 mgm. per day, surpassed that effected by the tyrosine presumably derived from ingested protein, together with a daily oral ration of 100 mgm. of

*l*-tyrosine. These contrasting effective dosages might be due to either defective absorption of orally administered tyrosine, to abnormal destruction of tyrosine in the intestine, or to an abnormality of utilization of absorbed tyrosine. No data are at hand which bear on these possibilities.

The *minimal quantities* of all four factors that induce a satisfactory response have not been determined. With Fraction E as the primary factor the smallest total dosage administered was 26 mgm. of solids per 10 days (Figure 2, Curve 18). With Fraction H as the primary factor the smallest dosage administered was 51 mgm. of solids, per 10 days (Figure 2, Curve 15).

The above quantities are of interest in relation to the purified liver extracts recently described by other workers. Very similar amounts, of total solids (per 10 days), with resulting satisfactory initial erythrocyte gains, were administered by Dakin, Ungley and West (9). On the other hand, Strandell, Poulsson and Schartum-Hansen (10) have recently reported hematopoietic responses following the use of purified amorphous material consisting of 0.35 mgm. of solids derived from 100 grams of liver, material that was prepared by Laland and Klem (11). Strandell and his collaborators administered this material to four patients, in the following dosages: Case I, total of 2.1 mgm. over a period of 42 days; Case II, 2.1 mgm. over a period of 32 days; Case III, 0.7 mgm. over a period of 9 days; and, Case IV, 0.7 mgm. over a period of 10 days. Although these authors conclude that 0.7 mgm. of their material "has a very good antianemic effect" their data, in our opinion, do not entirely substantiate this conclusion. Thus in Case I, although no initial erythrocyte count is given, on the seventh day the erythrocytes numbered 1.28 million, rising to 3.30 million at the end of 42 days, a distinctly submaximal response. In Case II the initial count is given as 0.91 million, rising to 2.1 million on the sixth day, but reaching only 2.45 million on the thirty-second day. In Case III the erythrocytes rose from 1.24 million to 1.4 million in 9 days. In Case IV the erythrocytes rose from 1.19 to 1.42 million in 10 days. These data, it seems to us, though indicating some therapeutic activity, are similar to the responses induced by our primary factor, in the absence of

the accessory factors (Figures 2 and 3). It has already been pointed out that the material of Laland and Klem shows certain chemical similarities to our Fraction I, derived from the primary factors, Fractions E or H (6).

#### SUMMARY AND CONCLUSIONS

Studies of the therapeutic activity of purified liver extract in pernicious anemia suggest that the hematopoietic effect may be exerted by an augmentative action of at least three chemically distinct *accessory* factors upon the activity of a *primary* factor. Of the three known accessory factors one is *l*-tyrosine, another contains a complex purine, and the third is a peptide. The accessory factors are completely devoid of the primary factor, and without the addition of the primary factor are therapeutically inert. The primary factor has been studied in an amorphous state. Its chemical nature is undetermined. Without the addition of the three accessory factors the primary factor is therapeutically only slightly active.

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# VITAMIN C SATURATION LEVELS IN THE BODY IN NORMAL SUBJECTS AND IN VARIOUS PATHOLOGICAL CONDITIONS<sup>1</sup>

By PHILIP FINKLE

(From the Division of Laboratories and from the Medical Service of Dr. George Baehr, the Mt. Sinai Hospital, New York City)

(Received for publication January 29, 1937)

There is still doubt concerning the rôle which vitamin C deficiency plays in pathological conditions, other than scurvy, which are characterized by vascular damage and a tendency to hemorrhage.

In a previous communication (2) the writer presented a preliminary report of studies upon urinary excretion of vitamin C in some vascular diseases, as well as in normal individuals. The rate of excretion in normal cases was found to be between 0.03 and 0.05 mgm. per cc. of urine during the day period (less during the night)—approximately that previously reported by Harris, Ray and Ward (1).

The present paper is concerned with further studies upon the utilization and excretion of vitamin C in conditions associated with vascular damage, hemorrhagic tendencies, as well as in other pathological states. One hundred and twenty-seven patients and normal controls were studied.

It seemed of interest to ascertain whether there is any change in the level of excretion in the normal individual when a quantity of vitamin C in excess of the usual average daily intake is administered. In order to eliminate the factor of variability in absorption or in destruction in the gastro-intestinal tract, the vitamin C was administered intravenously. This method was used in the work previously reported by the author (2).

## METHOD

In the earlier experiments presently reported the total 24 hour urinary excretion of vitamin C was first ascertained in each subject. On the following day, usually about 10 a.m., 100 mgm. of vitamin C (Merck) dissolved in 5 cc. of distilled water was injected intravenously, and again the 24 hour output of vitamin C in the urine was measured.

<sup>1</sup> Aided by a grant from Nelson I. Asiel. Aided, also, by a grant donated in memory of Solomon Sassoon Benjamin.

The results of these studies (Table I) indicate, 1, that the 24 hour output of vitamin C is usually about 13 to 20 mgm., and that the average is between 0.03 and 0.05 mgm. per cc. of urine during

TABLE I  
*Influence of vitamin C injection on vitamin C output*

Initials and remarks	Without intravenous vitamin C		On day of vitamin C injection	
	Total 24 hour output	Average output per day-time voiding	Output in 2 to 3 hours after injection	Total 24 hour output
	mgm.	mgm.	mgm.	mgm.
NORMAL INDIVIDUALS				
C. W.....	16.0	3.8	25.7	45.0
E. M.....	21.0	4.5	25.0	64.0
M. F.....	13.4	4.0	40.0	63.0
M. L.....	21.0	5.3	26.0	60.0
ABNORMAL INDIVIDUALS				
J. H. Bleeding peptic ulcer....	7.7	1.0	1.0	8.0
A. W. Gonorrheal arthritis....	9.0	1.6	2.5	13.0
D. T. Luetic aortitis.....	9.0	1.5	1.5	9.0
S. S. Carcinoma of bronchus..	9.1	1.1	2.1	9.3

the day period (being less during the night period). 2, In from 2 to 3 hours following the intravenous administration of 100 mgm. vitamin C, the excretion per cubic centimeter of urine rises to about 6 times the pre-injection level. The total 24 hour output rises to 45 to 65 mgm. vitamin C. This means that in addition to the usual daily output, 30 to 50 per cent (or more) of the vitamin C injected is excreted in the urine within the first 24 hours. 3, In those cases in which the output of vitamin C in individual voidings falls much below 3.0 mgm. the total output for the 24 hour period is considerably below the normal lev-



# VITAMIN C SATURATION LEVELS IN THE BODY IN NORMAL SUBJECTS AND IN VARIOUS PATHOLOGICAL CONDITIONS<sup>1</sup>

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There is still doubt concerning the rôle which vitamin C deficiency plays in pathological conditions, other than scurvy, which are characterized by vascular damage and a tendency to hemorrhage.

In a previous communication (2) the writer presented a preliminary report of studies upon urinary excretion of vitamin C in some vascular diseases, as well as in normal individuals. The rate of excretion in normal cases was found to be between 0.03 and 0.05 mgm. per cc. of urine during the day period (less during the night)—approximately that previously reported by Harris, Ray and Ward (1).

The present paper is concerned with further studies upon the utilization and excretion of vitamin C in conditions associated with vascular damage, hemorrhagic tendencies, as well as in other pathological states. One hundred and twenty-seven patients and normal controls were studied.

It seemed of interest to ascertain whether there is any change in the level of excretion in the normal individual when a quantity of vitamin C in excess of the usual average daily intake is administered. In order to eliminate the factor of variability in absorption or in destruction in the gastro-intestinal tract, the vitamin C was administered intravenously. This method was used in the work previously reported by the author (2).

## METHOD

In the earlier experiments presently reported the total 24 hour urinary excretion of vitamin C was first ascertained in each subject. On the following day, usually about 10 a.m., 100 mgm. of vitamin C (Merck) dissolved in 5 cc. of distilled water was injected intravenously, and again the 24 hour output of vitamin C in the urine was measured.

<sup>1</sup> Aided by a grant from Nelson I. Asiel. Aided, also, by a grant donated in memory of Solomon Sassoon Benjamin.

The results of these studies (Table I) indicate, 1, that the 24 hour output of vitamin C is usually about 13 to 20 mgm., and that the average is between 0.03 and 0.05 mgm. per cc. of urine during

TABLE I  
*Influence of vitamin C injection on vitamin C output*

Initials and remarks	Without intravenous vitamin C		On day of vitamin C injection	
	Total 24 hour output	Average output per day-time voiding	Output in 2 to 3 hours after injection	Total 24 hour output
	mgm.	mgm.	mgm.	mgm.
NORMAL INDIVIDUALS				
C. W.....	16.0	3.8	25.7	45.0
E. M.....	21.0	4.5	25.0	64.0
M. F.....	13.4	4.0	40.0	63.0
M. L.....	21.0	5.3	26.0	60.0
ABNORMAL INDIVIDUALS				
J. H. Bleeding peptic ulcer....	7.7	1.0	1.0	8.0
A. W. Gonorrheal arthritis....	9.0	1.6	2.5	13.0
D. T. Luetic aortitis.....	9.0	1.5	1.5	9.0
S. S. Carcinoma of bronchus..	9.1	1.1	2.1	9.3

the day period (being less during the night period). 2, In from 2 to 3 hours following the intravenous administration of 100 mgm. vitamin C, the excretion per cubic centimeter of urine rises to about 6 times the pre-injection level. The total 24 hour output rises to 45 to 65 mgm. vitamin C. This means that in addition to the usual daily output, 30 to 50 per cent (or more) of the vitamin C injected is excreted in the urine within the first 24 hours. 3, In those cases in which the output of vitamin C in individual voidings falls much below 3.0 mgm. the total output for the 24 hour period is considerably below the normal lev-



els. 4, When there is no acute rise in excretion following the intravenous administration of vitamin C, the total output for the 24 hour period remains at about the same low level as on the previous day, when no vitamin C was injected.

It was therefore considered unnecessary to continue to measure the total excretion of vitamin C for the entire 24 hour period. For the purposes of the investigation described here, the measurement of the total urinary excretion of vitamin C for about an 8 hour period during the day, including an approximately 6 hour period following intravenous injection, suffices to give a true indication of the state of saturation of the body with vitamin C.

To determine the state of body saturation with vitamin C the following routine was adopted by the present writer. On the day of the test the subject is permitted to have his or her usual meals, with the exception of citrus fruits and other foods containing vitamin C. There is no restriction of fluids. The urine is collected at about 9 a.m. and 11 a.m., and the vitamin C content determined in each specimen. After the 11 a.m. voiding, 100 mgm. vitamin C (cevitamic acid, or cebione, Merck) <sup>2</sup> dissolved in 5 cc. of distilled water was administered intravenously. The subject then was requested to void only at about 1:30, 3:30, and 5:30 p.m. respectively, and the vitamin C content was again determined. For the titration of cevitamic acid, 2:6-dichlorophenolindophenol as used by Harris, Ray and Ward (1) was employed. No untoward symptoms were noted, although more than 200 intravenous injections were given.

Since it was the purpose of this work to study subjects under normal conditions, no dietary restrictions were placed upon them other than those stated above.

The normal controls were chosen from among nurses, hospital internes, and laboratory technicians.

It is seen from Table II that in each of the 9 normal controls, following the intravenous administration of 100 mgm. of vitamin C, the excretion rises to about 4 to 8 times the values found before the injections.

<sup>2</sup> The author wishes to express his appreciation to Merck and Co. for their generous cooperation in supplying us with the ascorbic acid required in these studies.

TABLE II

*Influence of vitamin C injection on vitamin C output*

Name	Before Injection 6:30 to 11 a.m.	After injection	
		11 a.m. to 1:30 p.m.	1:30 to 5:30 p.m.
	mgm.	mgm.	mgm.
NORMAL CONTROLS			
E. M.	3.9	25.0	10.0
M. F.	6.0	40.	7.0
C. W.	3.8	25.7	4.3
D. S.	5.6	17.0	7.0
M. L.	5.3	26.0	5.0
A. F.	7.1	31.0	9.2
S. F.	3.2	24.0	6.0
F. M.	4.4	16.3	6.0
L. C.	5.1	10.2	4.8
PURPURAS			
Y. K. Purpura hemorrhagica	2.1	3.2	1.3
R. LaC. Purpura hemorrhagica	2.6	4.5	3.0
T. G. Purpura hemorrhagica	5.0	32.0	6.1
A. N. Purpura hemorrhagica	2.4	8.3	2.0
I. E. Purpura hemorrhagica	3.5	4.1	2.1
D. D. Purpura hemorrhagica	6.0	19.0	4.1
A. K. Purpura hemorrhagica	3.0	5.0	3.0
G. S. Purpura hemorrhagica	2.0	2.1	1.7
F. B. Purpura hemorrhagica	1.8	1.4	1.0
E. G. Purpura hemorrhagica	1.0	2.6	1.8
M. M. Purpura hemorrhagica	1.8	2.1	2.0
F. Gr. Purpura hemorrhagica	2.7	3.8	1.1
M. K. Purpura hemorrhagica	1.9	4.2	3.4
B. B. Purpura hemorrhagica	6.5	33.0	4.1
R. F. Purpura hemorrhagica	2.9	4.1	3.0
A. A. Purpura hemorrhagica	1.7	8.1	2.4
A. R. Purpura hemorrhagica	2.0	1.8	1.9
F. G. Purpura hemorrhagica	1.9	2.0	2.4
A. E. Purpura hemorrhagica	2.7	3.1	2.1
R. B. Symptomatic purpura	1.4	3.0	1.0
R. C. Symptomatic purpura	1.5	3.8	2.0
R. G. Symptomatic purpura	1.2	1.5	0.9
V. B. Hemophilia	3.6	5.3	3.3
J. K. Hemophilia	7.1	23.0	8.0
M. K. Hemophilia	3.9	38.2	5.3
E. Z. Metrorrhagia	3.5	6.2	1.7
M. L. Metrorrhagia	2.7	1.7	1.4
F. B. Metrorrhagia	2.1	3.2	1.8
R. B. Metrorrhagia	1.9	2.1	1.1
ACUTE LUPUS ERYTHEMATOSUS			
H. B.	1.0	2.1	1.5
M. G.	2.2	6.1	3.0
D. C.	1.1	2.0	0.8
L. N.	1.8	1.1	1.0
E. C.	2.0	2.1	1.8
NEPHRITIS			
M. U. Acute glomerular nephritis	1.4	12.2	2.0
P. R. Acute glomerular nephritis	3.1	11.1	1.3
E. C. Acute glomerular nephritis	1.0	1.6	1.5
A. D. Acute glomerular nephritis	0.9	2.6	1.1
W. O'B. Hypertensive	3.2	9.4	2.1
J. R. Hypertensive	5.0	20.5	6.8
H. R. Hypertensive	2.0	8.6	1.1
L. I. Hypertensive	2.3	3.9	3.6
B. M. Hypertensive	1.5	2.6	1.2
W. M. Hypertensive	1.3	1.4	1.9

TABLE II—Continued

Name	Before injection 6:30 to 11 a.m.	After injection	
		11 a.m. to 1:30 p.m.	1:30 to 5:30 p.m.
	mgm.	mgm.	mgm.
<b>ACUTE RHEUMATIC FEVER</b>			
D. R.	1.0	1.1	1.6
J. F.	1.9	2.3	1.2
J. V.	2.1	3.1	2.0
J. P.	2.0	4.1	2.2
G. G.	3.2	8.4	5.0
P. C.	2.0	9.1	1.8
R. M.	1.1	2.3	1.0
J. K.	2.7	5.3	3.1
H. Z.	1.7	6.1	2.9
N. F.	2.0	1.9	1.1
F. D.	2.8	19.1	2.0
M. S.	2.5	3.1	1.9
S. S.	4.4	20.2	3.8
M. H.	4.7	19.8	3.2
<b>RHEUMATOID ARTHRITIS</b>			
L. T.	2.1	3.0	1.8
D. H.	0.9	2.0	1.1
C. S.	2.8	11.3	1.9
M. Co.	3.1	13.4	2.0
C. S.	0.9	1.1	1.0
L. W.	7.1	23.0	4.4
M. D.	1.8	2.1	1.1
M. R.	3.4	4.1	2.8
A. C.	1.4	1.9	1.1
R. M.	1.4	1.9	0.9
E. McG.	1.1	2.3	1.4
M. P.	2.0	2.1	1.3
T. S.	5.1	18.4	3.6
J. R.	1.9	2.1	1.4
S. B.	2.3	1.4	1.8
C. F.	1.8	1.1	1.6
G. S.	1.4	2.0	0.8
F. B.	1.9	7.1	2.2
E. L.	1.0	1.2	1.1
A. R.	2.0	2.1	1.9
H. A.	1.7	2.0	1.1
I. S.	0.8	0.9	0.6
F. C.	1.4	1.1	0.9
J. S.	2.0	2.1	1.8
<b>JAUNDICE</b>			
M. S. Cinchophen	6.1	37.0	5.8
J. F. Catarrhal	3.9	8.4	4.4
M. C. Catarrhal	5.6	31.4	6.1
I. A. Catarrhal	1.8	3.2	1.1
T. R.	2.0	2.1	1.8
S. G.	1.1	1.3	1.0
H. S.	0.9	2.3	1.7
M. Cr.	6.3	31.2	5.8
F. S.	1.9	2.9	2.0
E. H.	2.4	3.1	1.8
H. Sa.	6.1	39.0	5.0
J. S. Post-arsenobenzol	1.1	2.6	1.4
L. O. Cinchophen	7.2	49.1	8.1
<b>MISCELLANEOUS GROUP</b>			
L. D. Adenoma of prostate	1.1	2.4	1.3
L. L.	5.5	21.4	4.7
E. G.	1.7	2.3	1.2
W. K.	1.7	2.4	1.1
G. G.	1.3	2.4	1.3
I. H.	1.3	2.9	1.5
<b>CLINIC CONTROLS</b>			
M. T. Non-infected dog bite	1.2	2.2	1.1
A. S. Small breast abscess	2.5	2.3	0.9
L. H. Lipoma of neck	0.8	1.5	1.0
M. C. Trauma right arm	1.4	1.8	1.1
J. G.	1.9	3.9	2.0
E. T. Luetic aortitis	1.3	3.0	1.7

The pre-injection values for vitamin C excretion given in the first column in Table II are the averages of the values for the two voidings prior to the intravenous injection.

From an average excretion of about 4.0 to 7.0 mgm. of vitamin C prior to intravenous injection, the excretion rises, within 2 to 3 hours after injection, to levels of from 16.0 to 40.0 mgm. of vitamin C. The normal level is again reached in about 4 to 6 hours.

### *The purpuras*

The knowledge that scurvy was associated with a dietary deficiency which may be corrected by the administration of lemons or limes antedates our knowledge of vitamins.

The isolation and synthesis of vitamin C stimulated a renewed interest in other conditions which, like scurvy, are associated with vascular damage and tendency to hemorrhage. The possibility suggested itself to Szent-Györgyi (5) and others that the purpuras might be associated etiologically with a vitamin C deficiency. Some observers have even used vitamin C as a therapeutic agent in purpura, and have reported good results.

Studies upon 29 cases of this type are recorded here. Included in this group are 19 cases of thrombocytopenic purpura, 3 cases of symptomatic purpura, and 3 cases of hemophilia. In addition, 4 cases of metrorrhagia in which no organic lesion could be demonstrated were studied with this group.

In comparing the values for vitamin C excretion in normal persons, with those found in the group of purpuras studied, a significant difference is found, Table II. With few exceptions the excretion is considerably lower in the purpuras than in normal individuals, being as low as 30 per cent of normal.

Following intravenous administration of cevitamic acid, only 3 of the 19 cases of purpura showed a normal rise in urinary excretion of vitamin C. The values for excretion still remained considerably below the normal levels in the remaining 16 cases.

It is noted that 2 of the 3 cases of hemophilia showed the normal rise in excretion following intravenous administration of vitamin C.

None of the cases of metrorrhagia showed any

cls. 4, When there is no acute rise in excretion following the intravenous administration of vitamin C, the total output for the 24 hour period remains at about the same low level as on the previous day, when no vitamin C was injected.

It was therefore considered unnecessary to continue to measure the total excretion of vitamin C for the entire 24 hour period. For the purposes of the investigation described here, the measurement of the total urinary excretion of vitamin C for about an 8 hour period during the day, including an approximately 6 hour period following intravenous injection, suffices to give a true indication of the state of saturation of the body with vitamin C.

To determine the state of body saturation with vitamin C the following routine was adopted by the present writer. On the day of the test the subject is permitted to have his or her usual meals, with the exception of citrus fruits and other foods containing vitamin C. There is no restriction of fluids. The urine is collected at about 9 a.m. and 11 a.m., and the vitamin C content determined in each specimen. After the 11 a.m. voiding, 100 mgm. vitamin C (cevitamic acid, or cebione, Merck)<sup>2</sup> dissolved in 5 cc. of distilled water was administered intravenously. The subject then was requested to void only at about 1:30, 3:30, and 5:30 p.m. respectively, and the vitamin C content was again determined. For the titration of cevitamic acid, 2:6-dichlorophenolindophenol as used by Harris, Ray and Ward (1) was employed. No untoward symptoms were noted, although more than 200 intravenous injections were given.

Since it was the purpose of this work to study subjects under normal conditions, no dietary restrictions were placed upon them other than those stated above.

The normal controls were chosen from among nurses, hospital internes, and laboratory technicians.

It is seen from Table II that in each of the 9 normal controls, following the intravenous administration of 100 mgm. of vitamin C, the excretion rises to about 4 to 8 times the values found before the injections.

<sup>2</sup> The author wishes to express his appreciation to Merck and Co. for their generous cooperation in supplying us with the ascorbic acid required in these studies.

TABLE II  
*Influence of vitamin C injection on vitamin C output*

Name	Before injection 6:30 to 11 a.m.	After injection	
		11 a.m. to 1:30 p.m.	1:30 to 5:30 p.m.
	mgm.	mgm.	mgm.
NORMAL CONTROLS			
E. M.	3.0	25.0	10.0
M. F.	6.0	40.	7.0
C. W.	3.8	25.7	4.3
D. S.	5.6	17.0	7.0
M. L.	5.3	26.0	5.0
A. F.	7.1	31.0	9.2
S. F.	3.2	24.0	6.0
F. M.	4.4	16.3	6.0
L. C.	5.1	10.2	4.8
PURPURAS			
Y. K. Purpura hemorrhagica	2.1	3.2	1.3
R. LaC. Purpura hemorrhagica	2.6	4.5	3.0
T. G. Purpura hemorrhagica	5.0	32.0	6.1
A. N. Purpura hemorrhagica	2.4	8.3	2.0
I. E. Purpura hemorrhagica	3.5	4.1	2.1
D. D. Purpura hemorrhagica	6.0	19.0	4.1
A. K. Purpura hemorrhagica	3.0	5.0	3.0
G. S. Purpura hemorrhagica	2.0	2.1	1.7
F. B. Purpura hemorrhagica	1.8	1.4	1.0
E. G. Purpura hemorrhagica	1.0	2.6	1.8
M. M. Purpura hemorrhagica	1.8	2.1	2.0
F. Gr. Purpura hemorrhagica	2.7	3.8	1.1
M. K. Purpura hemorrhagica	1.9	4.2	3.4
B. B. Purpura hemorrhagica	6.5	33.0	4.1
R. F. Purpura hemorrhagica	2.9	4.1	3.0
A. A. Purpura hemorrhagica	1.7	5.1	2.4
A. R. Purpura hemorrhagica	2.0	1.8	1.9
F. G. Purpura hemorrhagica	1.9	2.0	2.4
A. E. Purpura hemorrhagica	2.7	3.1	2.1
R. B. Symptomatic purpura	1.4	3.0	1.0
R. C. Symptomatic purpura	1.5	3.8	2.0
R. G. Symptomatic purpura	1.2	1.5	0.9
V. B. Hemophilia	3.6	5.3	3.3
J. K. Hemophilia	7.1	23.0	8.0
M. K. Hemophilia	3.9	38.2	5.3
E. Z. Metrorrhagia	3.5	6.2	1.7
M. L. Metrorrhagia	2.7	1.7	1.4
F. B. Metrorrhagia	2.1	3.2	1.8
R. B. Metrorrhagia	1.9	2.1	1.1
ACUTE LUPUS ERYTHEMATOSUS			
H. B.	1.0	2.1	1.5
M. G.	2.2	6.1	3.0
D. C.	1.1	2.0	0.8
L. N.	1.8	1.1	1.0
E. C.	2.0	2.1	1.8
NEPHRITIS			
M. U. Acute glomerular nephritis	1.4	12.2	2.0
P. R. Acute glomerular nephritis	3.1	11.1	1.3
E. C. Acute glomerular nephritis	1.0	1.6	1.5
A. D. Acute glomerular nephritis	0.9	2.0	1.1
W. O'B. Hypertensive	3.2	9.4	2.1
J. R. Hypertensive	5.0	20.5	6.8
H. R. Hypertensive	2.0	8.6	1.1
L. I. Hypertensive	2.3	3.9	3.6
B. M. Hypertensive	1.5	2.0	1.2
W. M. Hypertensive	1.3	1.4	1.9

TABLE 11—Continued

Name	Before injection 6:30 to 11 a.m.	After injection	
		11 a.m. to 1:30 p.m.	1:30 to 5:30 p.m.
	mgm.	mgm.	mgm.
ACUTE RHEUMATIC FEVER			
D. R.	1.0	1.1	1.6
J. F.	1.9	2.3	1.2
J. V.	2.1	9.1	2.0
J. P.	2.0	4.1	2.2
G. G.	3.2	8.4	5.0
P. C.	2.0	9.1	1.8
R. M.	1.1	2.3	1.0
J. K.	2.7	5.3	3.1
H. Z.	1.7	0.1	2.9
N. P.	2.0	1.0	1.1
F. D.	2.8	19.1	2.0
M. S.	2.5	3.1	1.9
S. S.	4.4	20.2	3.8
M. H.	4.7	19.8	3.2
RHEUMATOID ARTERITIS			
L. T.	2.1	3.0	1.8
D. H.	0.9	2.0	1.1
C. S.	2.8	11.3	1.9
M. Co.	3.1	13.4	2.0
C. S.	0.9	1.1	1.0
L. W.	7.1	23.0	4.4
M. D.	1.8	2.1	1.1
M. R.	3.4	4.1	2.8
A. C.	1.4	1.9	1.1
R. M.	1.4	1.9	0.9
E. McG.	1.1	2.3	1.4
M. P.	2.0	2.1	1.3
T. S.	5.1	18.4	3.6
J. R.	1.9	2.1	1.4
S. B.	2.3	1.4	1.8
C. F.	1.8	1.1	1.6
G. S.	1.4	2.0	0.8
F. B.	1.9	7.1	2.2
E. L.	1.0	1.2	1.1
A. R.	2.0	2.1	1.9
H. A.	1.7	2.0	1.1
I. S.	0.8	0.9	0.6
F. C.	1.4	1.1	0.9
J. S.	2.0	2.1	1.8
JAUNDICE			
M. S. Cinchophen.	6.1	37.0	5.8
J. F. Catarrhal.	3.9	8.4	4.4
M. C. Catarrhal.	5.6	31.4	6.1
I. A. Catarrhal.	1.8	3.2	1.1
T. R.	2.0	2.1	1.8
S. G.	1.1	1.3	1.0
H. S.	0.9	2.3	1.7
M. Cr.	6.3	31.2	5.8
F. S.	1.9	2.9	2.0
E. H.	2.4	3.1	1.8
H. Sa. Carcinoma of pancreas.	6.1	39.0	5.0
J. S. Post-arsenobenzol.	1.1	2.6	1.4
L. O. Cinchophen.	7.2	49.1	8.1
MISCELLANEOUS GROUP			
L. D. Addison's disease	1.1	2.4	1.3
L. L. Addison's disease	5.5	21.4	4.7
E. G.	1.7	2.3	1.2
W. K.	1.7	2.4	1.1
G. G.	1.3	2.4	1.3
I. H.	1.3	2.9	1.5
CLINIC CONTROLS			
M. T. Non-infected dog bite	1.2	2.2	1.1
A. S. Small breast abscess	2.5	2.3	0.9
L. H. Lipoma of neck	0.8	1.5	1.0
M. C. Trisuma right arm	1.4	1.8	1.1
J. G. Hernia	1.9	3.9	2.0
E. T. Luetic aortitis	1.3	3.0	1.7

The pre-injection values for vitamin C excretion given in the first column in Table II are the averages of the values for the two voidings prior to the intravenous injection.

From an average excretion of about 4.0 to 7.0 mgm. of vitamin C prior to intravenous injection, the excretion rises, within 2 to 3 hours after injection, to levels of from 16.0 to 40.0 mgm. of vitamin C. The normal level is again reached in about 4 to 6 hours.

### The purpuras

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The isolation and synthesis of vitamin C stimulated a renewed interest in other conditions which, like scurvy, are associated with vascular damage and tendency to hemorrhage. The possibility suggested itself to Szent-Györgyi (5) and others that the purpuras might be associated etiologically with a vitamin C deficiency. Some observers have even used vitamin C as a therapeutic agent in purpura, and have reported good results.

Studies upon 29 cases of this type are recorded here. Included in this group are 19 cases of thrombocytopenic purpura, 3 cases of symptomatic purpura, and 3 cases of hemophilia. In addition, 4 cases of metrorrhagia in which no organic lesion could be demonstrated were studied with this group.

In comparing the values for vitamin C excretion in normal persons, with those found in the group of purpuras studied, a significant difference is found, Table II. With few exceptions the excretion is considerably lower in the purpuras than in normal individuals, being as low as 30 per cent of normal.

Following intravenous administration of cevitamic acid, only 3 of the 19 cases of purpura showed a normal rise in urinary excretion of vitamin C. The values for excretion still remained considerably below the normal levels in the remaining 16 cases.

It is noted that 2 of the 3 cases of hemophilia showed the normal rise in excretion following intravenous administration of vitamin C.

None of the cases of metrorrhagia showed any

significant rise in excretion of vitamin C following intravenous administration of cevitic acid.

### *Acute lupus erythematosus*

Because of the tendency to petechial and purpuric hemorrhages, as well as the characteristic pathological changes found in the smaller blood vessels described by Baehr, Klemperer and Schiffrin (3) in cases of acute lupus erythematosus, it seemed possible that this group might in some manner be associated with disturbances in vitamin C metabolism.

Studies upon 5 cases of acute lupus erythematosus are here presented. All of these cases went to a fatal termination.

As seen from Table II, the average values for excretion of vitamin C in these cases was found to be between 1.0 and 2.0 mgm. vitamin C in each voiding, approximately 30 per cent of the values found in normal individuals. Following the intravenous administration of 100 mgm. of vitamin C there was no rise in the urinary output of vitamin C. The values still remained considerably below levels found in normal individuals before intravenous administration of cevitic acid.

### *Glomerulonephritis and hypertension*

In the cases of purpura and more particularly in the cases of acute lupus erythematosus there occurs not infrequently a considerable degree of vascular damage in the kidneys. The question arises whether the low excretion of vitamin C in these cases could be ascribed to diminished excretory activity of the damaged kidneys. Accordingly, a group of 10 cases of acute glomerulonephritis and hypertension with renal vascular involvement was studied. The results of these studies (Table II) indicate that even such severe damage to the capillaries of the kidneys as is found in cases of acute glomerulonephritis is not necessarily associated with any decreased permeability of the kidneys for vitamin C. In 5 of the 10 cases studied there is a rise in excretion of vitamin C following intravenous administration. In one of these 5 cases the rise is to 20.5 mgm. of vitamin C. In the other 4 cases the post-injection levels range from 8.6 to 12.2 mgm.

### *Rheumatic fever*

Rinehart et al. (4) suggested that a vitamin C deficiency might be one of the etiological factors in the development of acute rheumatic fever. It was therefore considered of interest to study the vitamin C excretion in a group of cases of *acute rheumatic fever*. In Table II there are presented the results of these studies upon 14 cases ranging in age from 12 to 49 years, and in varying stages of the disease. In 6 of the cases there is an increase in excretion of vitamin C following intravenous injection of 100 mgm. of ascorbic acid. While in some of these cases urinary excretion of vitamin C before intravenous injection is below normal, yet, following injection, there are increases in output ranging up to about 7 times the pre-injection level.

### *Rheumatoid arthritis*

In order to ascertain whether there is any significant difference in vitamin C metabolism between the cases of *acute rheumatic fever* and *rheumatoid arthritis*, 24 cases of the latter group were studied. From Table II it is seen that of the 24 cases studied, only 4 showed a normal vitamin C excretion curve in the urine. The remaining 20 cases either had excretion levels considerably below normal, or had no rise in excretion following intravenous administration of vitamin C.

### *Jaundice*

At present there is no satisfactory explanation for the enhanced tendency to bleed in cases of jaundice. The possibility suggests itself that in association with the greater or lesser degree of damage to the liver some disturbance in vitamin C metabolism might be concerned with the increased bleeding tendency. Studies of vitamin C excretion were therefore made upon a group of 13 cases of jaundice of various types. The results of these studies are given in Table II. In 5 of the cases, the vitamin C excretion test is apparently normal, while in the remaining 8 cases there is little or no rise in excretion following the intravenous injection. No uniform disturbance of vitamin C metabolism was demonstrable in these cases of jaundice.

In Table II are given of studies  
upon a group of 2 cases

of Addison's disease, 2 cases of ulcerative colitis, 1 case of peptic ulcer, and 1 case of non-tropical sprue. The vitamin C excretion tests in all but one case show a low vitamin C saturation. The exception is one of the cases of Addison's disease which has a normal excretion curve. A vitamin C deficiency could have been anticipated in the intestinal cases (including the case of sprue) as a result of the dietary restrictions imposed in these cases.

From the foregoing studies upon 115 subjects it is clear that abnormally low vitamin C excretion levels occur in a variety of pathological conditions. It should be evident, however, that the mere demonstration of an abnormally low vitamin C excretion level in a given disease is not adequate evidence that the disease is due to a vitamin C deficiency.

The high incidence of low vitamin C saturation in many of the patients studied leads one to suspect that a large percentage of the population from which these clinic and ward patients are drawn is on a low vitamin C intake as a part of a general dietetic deficiency. It was therefore considered desirable to study a group of patients from departments such as the orthopedic clinics and the surgical clinics, in order to ascertain whether they show any vitamin C deficiency as indicated by a low excretion level.

Six patients were selected at random from the traumatic surgical clinic. These patients were chosen from a group which had no demonstrable vascular disease or tendency to hemorrhage, the conditions being fractures, other trauma, dog bite, etc. From Table II it can be seen that all of these patients have low excretion levels of vitamin C in the urine, with practically no rise following intravenous injection. These patients had a low vitamin C saturation level, although they had no clinical manifestations which could in any way be associated with a vitamin C deficiency.

*Observations following administration of orange juice to individuals showing a low level of vitamin C saturation*

The question arises whether the administration of vitamin C in the form of orange juice would suffice to remedy this subclinical degree of deficiency found in such a variety of conditions. In

TABLE 111  
*Influence of vitamin C injection on vitamin C output*

Name	Before course of orange juice			After course of orange juice		
	Before injection, 6:30 to 11 a.m.	After injection		Before injection, 6:30 to 11 a.m.	After injection	
		11 a.m. to 1:30 p.m.	1:30 to 5:30 p.m.		11 a.m. to 1:30 p.m.	1:30 to 5:30 p.m.
	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
M. T. Acute glomerular nephritis..	1.5	3.1	1.1	5.3	20.2	4.8
V. R. Acute glomerular nephritis..	2.0	3.6	1.4	5.0	17.6	4.1
M. K. Purpura hemorrhagica. ....	1.9	4.2	3.4	4.9	41.0	5.1
A. A. " "	1.7	8.1	2.4	3.1	19.0	1.8
G. G. " "	1.3	2.4	1.3	6.0	31.6	5.8
E. T. " "	1.3	3.0	1.7	7.0	40.2	6.8
E. F. " "	1.2	3.0	1.3	18.0	75.2	9.8
E. C. Acute lupus erythematosus .				2.2	2.9	1.9
H. B. Acute lupus erythematosus .				2.4	6.1	2.3
M. G. Acute lupus erythematosus .				2.2	6.1	2.0

Table III are shown the results of the administration of from 200 to 400 cc. of orange juice daily for 1 week to 7 patients. The saturation test in each of these cases shows practically no rise in excretion of vitamin C before the patients received orange juice. Following the administration of orange juice for 1 week, the saturation curve in each of the 7 cases becomes normal. This indicates that these subjects became normally saturated with vitamin C.

Similar study of 3 of the cases of acute lupus erythematosus discloses a surprisingly different picture. Table III shows the results of these studies. One patient, M. G., had been receiving a high vitamin C diet including orange juice and grapefruit for more than two weeks. The saturation test shows practically no rise in excretion following intravenous administration.

Another patient, H. B., received 200 mgm. cevitamic acid intravenously every day for 6 days, and was then given about 500 cc. of orange juice daily for more than 2 weeks. The vitamin C saturation test showed no rise in excretion, as shown in Table III.

The third case, E. C., had been taking more than ordinarily adequate quantities of orange juice daily for several weeks. Nevertheless, the vitamin C saturation test showed an abnormally low level of excretion.

In the cases of lupus erythematosus, the vitamin C deficiency is apparently not remedied in the same manner as in the other types of cases stud-

ied—that is, by saturating the patient with orange juice or administering cevitamic acid intravenously.

#### DISCUSSION

In an attempt to elucidate the problems of vitamin C metabolism it has been the object of these studies to answer, if possible, the following questions: 1. Is there a relatively constant level of urinary excretion of vitamin C in normal individuals on what is considered a well balanced diet? 2. Is there a change in the excretion level, following the administration of a definite amount of vitamin C to the normal subject? 3. Under what conditions is the level of excretion significantly above or below the normal, and what is the response in such conditions to the administration of a similar quantity of vitamin C? 4. Do individuals with hemorrhagic tendencies have characteristically abnormal vitamin C excretion levels? 5. Are there other pathological conditions which are etiologically in some manner associated with an abnormal vitamin C excretion level?

From the work of Harris et al. (1) and the corroborating results of this and the previous study (2), it would appear that in normals on the conventional well balanced diet, there is a reasonably constant level of urinary excretion of vitamin C.

The work presented in this paper and also in a previous communication (2) establishes, furthermore, that normally there is a characteristic rise in excretion of vitamin C in the urine following the intravenous administration of 100 mgm. of vitamin C (cevitamic acid). The excretion level rises within about 2 hours, to about 5 to 6 times the values found before injection. There is a return to the normal level in from 4 to 6 hours following the injection. Because the excretion in these subjects rises to a peak following intravenous injection, with a later fall to pre-injection level, it may reasonably be assumed that the vitamin C in their body is at a definite saturation level.

These studies indicate also that there is a very large group in the ward and outpatient department population whose urinary excretion level of vitamin C is considerably below the normal. It is probable that these patients are representative of their group in the population.

When 100 mgm. of cevitamic acid is administered intravenously to any of this group, a rise in excretion fails to occur. This, in the opinion of the writer, is due to the fact that their vitamin C level is considerably below the normal saturation level—that there is a vitamin C deficiency. For that reason the test described here is designated the vitamin C saturation test. Included in this group are persons with many types of clinical conditions which cannot be associated causally with a vitamin C deficiency.

Since the isolation of vitamin C, many papers have been written in an effort to prove an etiological relationship between vitamin C deficiency and hemorrhagic diatheses such as purpura. Many claims of improvement or cure of purpura following treatment with vitamin C have been made on rather questionable evidence. The work presented in this paper indicates that in purpura, as well as in other conditions, there is an abnormally low level of vitamin C in the body and therefore a low level of excretion after intravenous injection of a test dose. This degree of vitamin C deficiency is, as would appear from this study, not peculiar to purpura. It is questionable, therefore, whether this vitamin C deficiency is an important etiological factor in the development of purpura—and it is extremely unlikely that it can be the sole etiological factor. This under-saturation with vitamin C is quickly remedied when orange juice is administered to these persons for a few days. This is shown by the fact that following intravenous injection of the test dose, the urinary excretion curve subsequently becomes normal.

The conclusion seems inescapable that a fairly large proportion of the population, as represented by the groups encountered in the wards and outpatient department of our hospital, suffers from an undersaturation with vitamin C. This might be termed a subclinical vitamin C deficiency. In most instances this is, undoubtedly, merely part of a general dietary deficiency.

It is not irrelevant, therefore, to sound a warning note to those who in their enthusiasm might misinterpret the significance of this degree of vitamin C deficiency in patients with purpura and other pathological conditions. Thus far the etiological rôle of vitamin C deficiency has been dem-



onstrated in the case of scurvy only. There is not sufficient evidence to indicate that it alone plays a rôle in any other pathological condition characterized by bleeding tendency or by vascular disease.

From the present studies there is a suggestion that the cases of acute lupus erythematosus may be associated with some disturbance in vitamin C metabolism. The 5 cases studied show a persistently low level of vitamin C excretion. Although 3 of these cases received an ordinarily sufficient quantity of orange juice to bring the saturation level to normal, these patients failed to respond in the same manner as did other patients thus treated. When patients with purpura and other conditions received orange juice daily for 1 week, the vitamin C saturation in the body became normal, as indicated by the test. Of the 3 cases of acute lupus erythematosus, one received large amounts of orange juice daily for several weeks; a second of these received cevitic acid intravenously for several days, followed by about 500 cc. of orange juice daily for some weeks; and the third one had been receiving one or more oranges daily for six weeks. Quantities of vitamin C in excess of the amounts apparently sufficient to overcome vitamin C deficiency in all other pathological conditions studied did not suffice to accomplish such a result in these 3 cases of acute lupus erythematosus.

The significance, if any, of this failure of the cases of acute lupus erythematosus to respond as do other cases cannot be appraised without further work. The daily administration of orange juice and the daily injection of cevitic acid was not followed by any improvement in the disease.

#### SUMMARY

In the present paper are presented the results of studies of vitamin C saturation and excretion in the urine upon 127 subjects. Besides normal controls, the cases studied included purpura (both thrombocytopenic and symptomatic), acute lupus erythematosus, glomerulonephritis, acute rheumatic fever, rheumatoid arthritis, and jaundice of varied etiology.

1. A test for vitamin C saturation or subclinical deficiency is presented.

2. In normal individuals the daily (24 hour) output of vitamin C is about 13 to 20 mgm.

3. When 100 mgm. of vitamin C is injected intravenously in normal individuals there is a rise in output, within 2 to 3 hours, to an average of about 5 times the pre-injection levels. The peak of post-injection excretion of vitamin C reaches a level equal to, or considerably above the 13 to 20 mgm. excreted normally in 24 hours, when no cevitic acid has been administered intravenously.

4. A large percentage of the population encountered in the wards and outpatient department of the Hospital were found to have excretion levels of vitamin C in the urine considerably below that found in normal subjects living on an adequate mixed diet. When such individuals receive 100 mgm. of vitamin C intravenously, there is practically no rise in urinary excretion of vitamin C. Similarly, degrees of subclinical deficiency could be demonstrated in purpura, and in patients with a variety of other diseases which were corrected by an adequate intake of orange juice for several days.

5. It is therefore maintained that there is, as yet, no evidence to justify a conclusion that vitamin C deficiency has a causal relationship to any pathological condition other than scurvy. A low excretion level of vitamin C does not warrant the conclusion that vitamin C deficiency plays an etiological rôle in thrombocytopenic purpura or other conditions which manifest hemorrhagic tendencies.

6. The persistently low excretion level of vitamin C in acute lupus erythematosus differs from that found in any other condition studied.

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# STUDIES ON THE ELASTIC PROPERTIES OF HUMAN ISOLATED AORTA

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(Received for publication February 11, 1937)

## THE VOLUME-ELASTICITY RELATIONS OF THE AORTA TO PRESSURE AND AGE

For the most part the study of the physiology of circulation has been confined to the heart and to a somewhat lesser extent to the peripheral blood vessels. The importance of the large central vessels as a functional unit of the cardiovascular apparatus has not been fully appreciated, chiefly because they have been looked upon merely as channels for the passage of blood from the heart to the periphery. But their relationship to the cardiovascular apparatus extends beyond this conception. By virtue of its inherent elastic properties, the aorta and its main branches become an essential functional unit of the circulatory system and contribute to an appreciable extent in maintaining the efficiency of the circulation. We have sought in this present investigation to study the elasticity characteristics of isolated human aortas with a view of determining the effect of these responses to the work of the heart and, further, to obtain quantitative data regarding the readjustments that may occur in the aorta under conditions of hypertension and arteriosclerosis.

Roy (1) in 1880, studied the relation between the internal pressure and the volumetric capacity of arteries by making direct physical measurements on isolated aortas of animals. By means of a series of curves, he showed that the aortic walls were most extensible at pressures corresponding (approximately) to their normal blood pressures, and that at higher pressures the extensibility of the wall of the vessel was considerably impaired. In making his observations, he allowed 20 to 30 minutes for the intra-arterial pressure to rise from 0 to 200 mm. Hg. The reason for making his observation slowly, was to reduce to a minimum any influence due to elastic "after-action"—the property of elastic tissue such as arteries to continue expanding for some time if the pressure or tension be constantly main-

tained. Bramwell and Hill (2), in 1922, after measuring the slopes at various points on Roy's curves, calculated the velocity of the pulse wave at various pressures. They found that the velocities, at pressures of 80 mm. Hg (equivalent to normal diastolic pressure in man), obtained from Roy's work were lower than those observed in man. Bramwell and Hill (2) in discussing Roy's method, stated, "From a point of view of static effect of the diastolic pressure of the arteries, he (Roy) succeeded; from that, however, of the dynamic effects occurring in a rapid cycle of events associated with the pulse, his precautions aggravated the error and must have caused the increase of volume per millimeter of mercury to be much larger than that occurring in a rapid change of pressure." They believed, therefore, that the velocities calculated from Roy's work were low because of the effect of elastic "after-action." To eliminate the possibility of elastic "after-action," these investigators devised a means of measuring the pulse wave velocity directly on isolated arteries (common carotid), and their results compared quite favorably with those observed in man. The vessels used in their study showed no microscopic postmortem evidence of arterial disease. Bramwell, Downing and Hill (3), in an attempt to arrive indirectly at a qualitative picture of arterial rigidity by numerical integration, constructed curves from their data relating total volume of an artery to the pressure inside it. They abandoned Roy's method because it did not measure the sharp momentary increase of volume such as follows the heart beat. They obtained their mean normal curve from a set of curves representing six normal individuals in the ages ranging from 8 to 45 years.

In this study, in order to obtain characteristic curves for the various age groups instead of one curve for the combined ages, the volume-elasticity characteristics of eighteen aortas were experi-



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In this study, in order to obtain characteristic curves for the various age groups instead of one curve for the combined ages, the volume-elasticity characteristics of eighteen aortas were experi-

(4). In addition to the lack of elastic tissue in aortas of this group, the extensibility of the vessels is further hampered by the increase of collagenous fibers (senile fibrosis) and by depositions of arteriosclerotic products in the vessel wall.

Since the aortic chamber is essentially an elastic structure as indicated by Figure 2, it follows that the aorta as well as its main branches constitute an important dynamic unit of the cardiovascular apparatus. Thus, as a result of its elastic retraction during the phase of cardiac diastole, the aortic chamber functions in the capacity of a buffer system converting a cardiac outflow which would be otherwise intermittent into one which is continuous in the capillaries.

In addition to its influence on the peripheral circulation, the aorta tends to minimize the work of the heart by facilitating the discharge of blood from the left ventricle, as pointed out by Bramwell. Such a view is indeed tenable when one considers the volume-elasticity curves as exhibiting the relation which exists between the pressure in the aorta and its ability to accept the cardiac output. It will be noted from the volume-elasticity curves *a*, *b*, and *c*, that within the range of normal diastolic pressure only a slight elevation of pressure is required to produce a considerable increase of volume, thus indicating the ease by which the elastic aorta can accept its ventricular outflow, providing of course other factors remain normal. But with increasing pressure, the trend of these curves is to flatten out showing that the aortic reservoir can no longer accommodate the same volume of blood with the same small pressure increase as it formerly could. On the contrary, a much greater rise in pressure is required to receive the same output. If, for example, we consider Curve *c*, it will be noted that at a diastolic pressure of 75 mm. Hg a rise of 15 mm. of pressure will produce a 17 per cent increase in volume; while at 125 mm. of pressure, a rise of 15 mm. will produce only a 7 per cent increase of volume; and at 150 mm. pressure, an increase of 15 mm. will only bring about a 6 per cent increase of volume. In other words, to produce a 15 per cent increase of volume at 150 mm. pressure, an increase of 50 mm. would be required. This readjustment could only be brought about

by an increased effort on the part of the heart in an attempt to augment the pulse pressure. Thus the mechanical effectiveness of the aortic chamber in receiving the cardiac output and passing it on to the peripheral circulation diminishes progressively with increasing pressures. From this it can readily be understood why the elasticity response of the aorta is physically hampered in cases of hypertension associated with high diastolic pressures.

The effect of diminution in arterial elasticity associated with advancing years is demonstrated by Curve *c*. Even within the range of normal diastolic pressures, the curve clearly shows a comparatively limited degree of elasticity response. It is obvious that the coefficient of elasticity is greatly altered with age. For unit increase in pressure the aorta from younger persons is distended much more than the aorta from individuals of older age groups.

However, the absolute volume of the aorta per unit of length is greater under conditions of low pressure in the older than it is in the younger age groups. Figure 3 illustrates this situation for the eighteen aortas studied completely. Several interesting points are apparent in this graph. The aortas of age group 20 to 24 years (Curve *a*) show much smaller volumes at 100 mm. Hg pressure than do those of ages 71 to 78 years (Curve *c*). Yet the volumes at higher pressures (200 to 225 mm. Hg) are considerably greater in the young than in the old aortas. Thus, the greater extensibility of the younger aorta permits greater dilation under hypertensive conditions than does the older.

The high absolute volume (and diameter) of the older aorta at pressures around 100 mm. Hg is obviously fortunate. The work of the heart is less when the diameter of the aorta is great because less work has to be done in giving the blood velocity. The decreased extensibility at higher pressures (for the old as compared with the younger aorta) is apparently an unfavorable change.

The data can be presented in another way by plotting the volume change of the aorta between systolic and diastolic pressure levels. This is a change in aortic pulse volume. If one takes the change occurring between 75 and 125 mm. Hg,

which is the "normal type" of pulse pressure relation, and also the volume change occurring between 125 and 225 mm. Hg, which approximates a typical "hypertensive" pressure situation

usually less under hypertensive conditions than under normal pressure conditions, although the pulse pressure was taken as 100 mm. Hg, or twice the normal in the hypertensive situation. This is

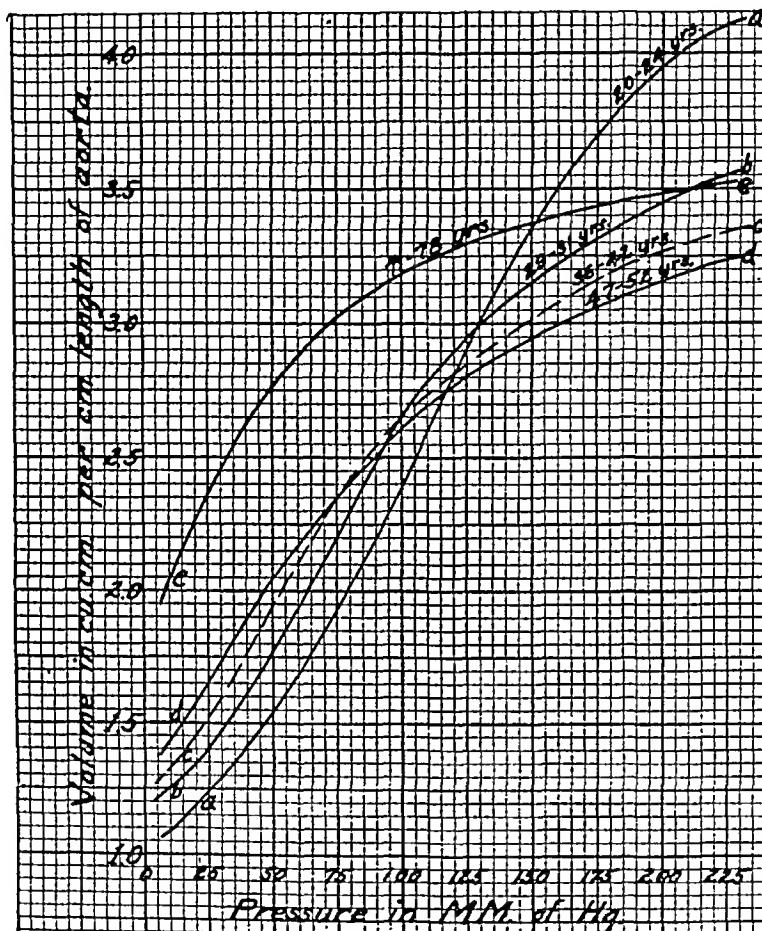


FIG. 3. CURVES SHOWING THE ABSOLUTE VOLUME PER UNIT LENGTH OF AORTA AT PRESSURES UP TO 230 MM. HG

It will be noted that the senile aorta has a relatively large volume at normal pressures and that the younger aorta has a greater volume at hypertensive pressures.

in diastole and systole respectively, one can observe certain important facts. Figure 4 presents this data graphically. It can be seen that the "pulse volume change" of the aorta diminishes to 25 per cent or less of that found in the young by the time age 75 has been reached. The aorta no longer acts as an elastic reservoir whose recoil can keep up the pressure and the flow in diastole. Furthermore, and this might not have been predicted, the aortic "pulse volume change" is ac-

true because the volume-elasticity curve (see Figure 2) is so nearly flat at the higher pressures in the older aortas. Thus, not only does the aorta become inferior as an elastic reservoir with age, but the hypertension itself makes the aorta function over a range of pressures over which its volume change is less than it would be with pulse pressures half as great at normal systolic pressures. The arteriosclerotic aorta would apparently function better at low pressures.

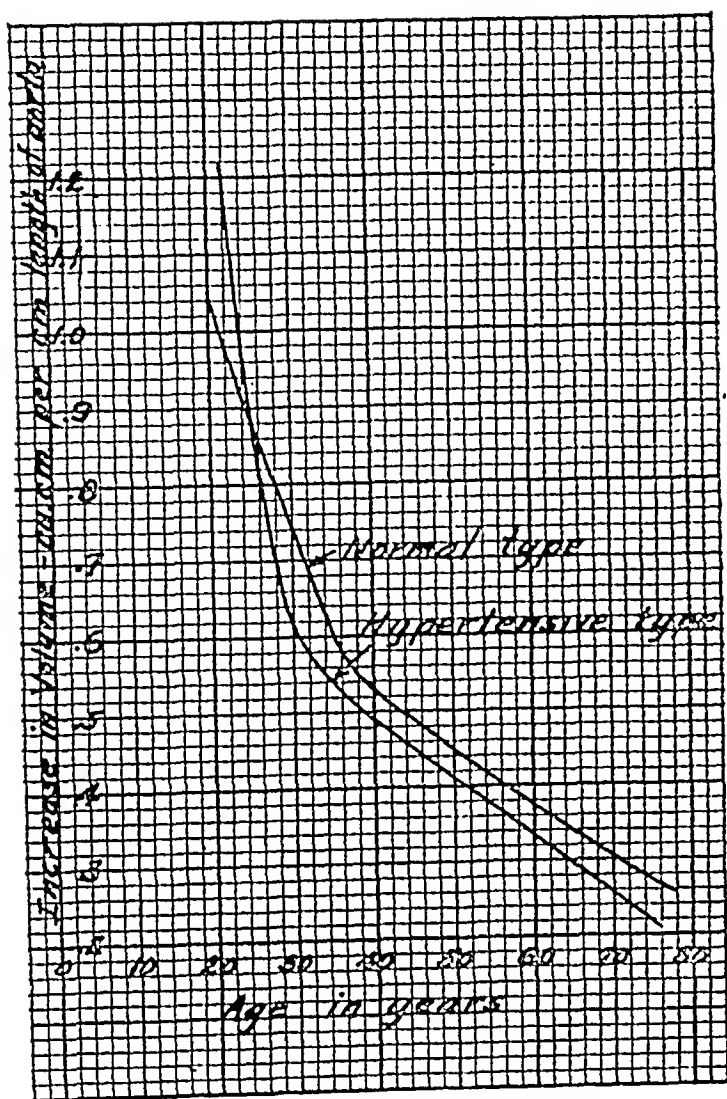


FIG. 4. CURVES SHOWING THE CHANGE IN PULSE VOLUME WITH INCREASING AGE FOR BOTH NORMAL AND HYPERTENSIVE PULSE PRESSURES (50 AND 100 MM. Hg RESPECTIVELY)

Anatomical studies of the elastic arteries in the senile period show that the aorta increases in length as well as in diameter. There is a marked lack of uniformity in length and diameter in the aortas of the senile period. A large proportion show marked ectatic dilatation while in others the dilatation is not so pronounced. Suffice it to say that they all show a definite longitudinal as well as a transverse enlargement of various degrees. Thinning of the aortic wall accompanies this alteration. Thus it is evident that the aorta can accommodate a greater volume of blood in its resting state than can the aorta of the younger age groups. What functional capacity the senile aorta loses by way of diminution in its elastic tissue is compensated for to a large extent, by an increase in its size. In other words, the senile

aorta becomes essentially a capacity chamber and does not need to be distended in order to accommodate the systolic discharge of the left ventricle without the production of very high velocities of flow. Instead, due to the increased diameter, the stroke output can be carried away at relatively low velocities and therefore with relatively low energy expenditure of the heart.

The above alteration that occurs in the aortas of the older age group should be looked upon as a normal, as well as a favorable, physiological adjustment to the ageing process, whereby the deleterious effect of increasing rigidity of the aorta and its main branches upon the heart is prevented. This accounts for the fact that the so-called "uncoiling" of the aorta (elongation and dilatation of this vessel) in the absence of hypertension is, as a rule, associated with hearts of normal size in elderly people (9, 10).

#### A COMPARISON OF THE EXTENSIBILITY OF LIVING AORTA WITH THAT OF ISOLATED AORTA

As shown by Bramwell and Hill (2), the pulse wave velocity may be derived from the Moen equation and expressed in a convenient formula:

$$V = \frac{3.57}{\sqrt{(\text{percentage increase in volume per mm. Hg increase of pressure})}},$$

in which  $V$  is the velocity of the front of the pulse wave in terms of meters per second. Therefore, it is possible to calculate the velocity of transmission of the pulse wave from the mean volume-elasticity curves for the designated ages and thereby compare the pulse wave velocity of vessels *in vivo* with those of isolated ones. The pulse wave velocities given below were calculated from the tangents to the respective curves (Figure 2) after being reconstructed on the basis of the normal volumes, assuming a pressure of 85 mm. Hg. Obviously, the volume at zero pressure cannot be used as a basis for calculation since that volume does not correspond to the volume *in vivo*. Hence, the normal volume of the aorta at normal internal pressure must be used to calculate the pulse wave velocity since that is the volume which varies in accordance with pressure changes occurring in the aortic chamber in life.

TABLE I

*Calculated and optically-measured pulse wave velocities, in terms of meters per second, and the resulting percentage differences for the various age groups*

Age groups	Velocity calculated from volume-elasticity curves of isolated aortas	Velocity in vivo by optical recording (7) *	Velocity in vivo by optical recording minus 0.4 meters	Difference	Percentage difference
<i>years</i>					<i>per cent</i>
20-24	4.3	5.2	4.8	0.5	10.4
29-31	5.0	5.6	5.2	0.2	3.9
36-42	5.8	6.4	6.0	0.2	3.3
47-52	6.5	7.5	7.1	0.6	8.4
71-78	9.5	10.5	10.1	0.6	5.9

Average percentage difference = 6.4 per cent

\* These values represent the mean pulse wave velocities of aortas for the various ages as obtained from a study of 550 normal living subjects. See reference 7 of the bibliography.

In Table I, the calculated velocity for each of the age groups is given in the second column. The velocity obtained by the optical recording method (7), and the velocity after subtracting 0.4 meter per second, are given in the third and fourth columns respectively. Obviously, the velocity obtained by the optical recording method includes the velocity of the blood flow itself which is not present nor accounted for in the velocity calculated from the volume-elasticity curves. This velocity of blood flow is approximately 0.4 meter per second (11) in the aorta, which value must be subtracted before comparing with the calculated velocity. The difference between this resulting velocity and the calculated velocity is given in the fifth column, and the percentage difference of the measured velocity is given in the last column. The agreement between the values for the actual pulse wave velocity measured on living persons and that calculated from the volume-elasticity curves is good, namely, 6.4 per cent average percentage difference. Incidentally, the percentage differences with increasing age show a striking constancy (about 6.4 per cent), thus indicating the accuracy as well as the validity of Bramwell and Hill's formula.

Another factor, the effect of elastic "after-action," tends to produce slower calculated velocities in isolated vessels. By using the described experimental method, this factor is almost but not entirely eliminated. In measuring the volume of

the isolated aorta, we found that at the same initial pressures before and after the experiment, the volume after the successive increments of pressure were applied, was increased from 0.75 to 1.0 cubic centimeter or about 8 per cent greater than the original volume, especially in the aortas of older subjects. Since the isolated aortas cannot "recoil" to its original or normal diameter, a greater increase in volume would be produced by this method than if completely instantaneous readings could have been made. Hence a 4 per cent slower calculated pulse-wave velocity results. Taking this into consideration and correcting the velocities determined from volume-elasticity curves, the data of Table II is obtained.

TABLE II

*Calculated pulse wave velocities corrected for elastic "after-action," optically-measured velocities, in terms of meters per second, and the resulting percentage differences for the various age groups*

Age groups	Calculated velocity corrected for 8 per cent "elastic after-action"	Mean velocities measured in vivo (Table I corrected for velocity of blood flow)	Differences	Percentage difference
<i>years</i>				<i>per cent</i>
20-24	4.5	4.8	0.3	6.2
29-31	5.2	5.2	0.0	0.0
36-42	6.0	6.0	0.0	0.0
47-52	6.8	7.1	0.3	5.0
71-78	9.9	10.1	0.2	2.0

Average percentage difference = 2.6 per cent

There are several other factors that may account for the 2.6 per cent difference which still exists. It must be remembered that the aorta is surrounded in the living man by a considerable amount of dense fibrous tissue. In all probability this tends to increase the rigidity of the arterial wall somewhat and thus increase the velocity of the pulse wave, whereas in the isolated aortic segment, the layer of dense fibrous tissue was removed in order to ligate the bases of the intercostal vessels. No exact physical measurements are yet available which demonstrate what effect this coating of fibrous tissue has on the extensibility of the aortic chamber. Another factor that may account in part for the abovementioned difference is the actual diastolic pressure present in the aorta. The above tabulated velocities for isolated aortas were computed on a basis of 85



mm. Hg pressure. It is obviously impossible to obtain the exact diastolic pressure in the living aorta. Undoubtedly it is higher than the normal diastolic pressure as measured over the brachial artery.

Aside from the factors just discussed, it is evident that the velocities of the pulse wave as calculated from volume-elasticity measurements on the isolated aorta are in substantial agreement with that obtained by the optical recording method *in vivo*.

#### CONCLUSIONS

1. A method for experimentally obtaining the volume-elasticity coefficients of isolated aortas has been described.

2. The mean volume-elasticity curves demonstrate that arterial rigidity increases, (*a*) progressively with age and (*b*) with increasing diastolic pressure.

3. The aorta of old age assumes the rôle of a capacity chamber or reservoir and by virtue of this readjustment, it becomes adapted to the reception of the cardiac output without imposing an undue strain on the heart, a condition which would otherwise result if the disappearance of elastic tissue occurred without a concomitant increase in the diameter and length of the aorta.

4. If the pulse wave velocities are calculated from mean volume-elasticity curves and compared with the mean pulse wave velocities obtained in living man at corresponding ages, it is found that the mean values obtained on the isolated aorta are less than those obtained *in vivo* by about 6 per cent. In the study of isolated aortas, the velocity

values are slightly low due largely to the factor of elastic "after-action."

5. Due to the satisfactory agreement of the pulse wave velocities found *in vivo* and in the isolated aortas it is possible to evaluate the condition of the aorta in living man by comparison with an isolated aorta having approximately the same pulse wave velocity.

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# CALCIUM AND PHOSPHORUS METABOLISM IN OSTEOMALACIA.

## V. THE EFFECT OF VARYING LEVELS AND RATIOS OF CALCIUM TO PHOSPHORUS INTAKE ON THEIR SERUM LEVELS, PATHS OF EXCRETION AND BALANCES, IN THE PRESENCE OF CONTINUOUS VITAMIN D THERAPY

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Osteomalacia is a bone disease more commonly seen and with greater clinical implications in North China than elsewhere (1, 2, 3). The principal cause of the skeletal demineralization resides in vitamin D deficiency, a combination of its lack in the diet and exclusion of sunlight. By reason of such deficiency, calcium given by mouth fails to be absorbed. Poor intestinal absorption rather than excessive elimination is incriminated because it has been demonstrated by the studies of Hannon et al. (4) that the endogenous calcium metabolism in patients with osteomalacia on low intake is within normal limits and that calcium administered parenterally is largely retained. Under such circumstances while the endogenous destructive activity in the bones may not be excessive, the reparative process is very much interfered with through defective intestinal absorption so that skeletal decalcification inevitably ensues. The limited intake of calcium in common Chinese dietaries (5), and periods of mineral stress incident to pregnancy and lactation are some of the contributing factors that enter into the pathogenesis of osteomalacia.

Studies of the effect of vitamin D in the treatment of osteomalacia (4, 6) demonstrate the remarkable conserving action of vitamin D on calcium and phosphorus metabolism. As a result of its administration, intestinal absorption is promoted and endogenous elimination is decreased so that large quantities of calcium and phosphorus are available for deposition in the bones. The actual amount of calcium and phosphorus retained depends upon the level and ratio of intake of these elements. It has been shown in two patients with osteomalacia undergoing reparation initiated by vitamin D (7) that calcium retention varied directly with calcium intake while phosphorus retention was limited by both calcium and phos-

phorus intake. Fecal calcium likewise varied directly with calcium intake while fecal phosphorus was parallel with both calcium and phosphorus intake. When calcium supply is limited in relation to phosphorus (low Ca:P ratio) practically all the calcium absorbed is deposited, none appearing in the urine. On the other hand, when phosphorus supply is short compared with calcium (high Ca:P ratio), all the available phosphorus is retained and urinary phosphorus vanishes. Conservation of excretion through the urinary tract and efficient absorption through the intestinal canal account for the markedly positive balances in osteomalacia when reparation is brought about under the influence of vitamin D.

Similar observations on the effects of variations of the levels and ratios of calcium to phosphorus intake on their serum levels, paths of excretion and balances have been made on another patient with healing osteomalacia. But in contrast to the previous patients who received vitamin D only prior to the observations, the present subject was given vitamin D throughout the entire study so as to obviate any uncertainty in ascribing the metabolic results obtained to vitamin D action. Moreover, attempt was made in the present study to secure more nearly metabolic equilibrium by using three 4-day periods for each level or ratio of dietary intake. The data obtained from this patient, together with those from another subject having syphilitic osteitis of right radius and tibia without general metabolic disturbance, taken as a control, constitute the basis of discussion in the present communication.

### PROCEDURE

The clinical histories of the two subjects are briefly described in the appendix. Subject 1, H. F. M., was a woman of 32 with advanced osteo-

malacia of seven years' duration. While skeletal rarefaction and deformities were marked, her serum calcium and phosphorus were within normal limits. She was placed on various diets, the compositions of which are given in Table I. All the diets were low in calcium but contained varying amounts of phosphorus. The desired high levels of calcium intake were attained by giving appropriate quantities of a saturated solution of calcium lactate (7.7 per cent). At a given level of calcium intake, the phosphorus level was progressively increased by giving Diets 5, 2, 3 and 4 in that order. There were altogether 3 levels of calcium and 4 levels of phosphorus intake, making a total of 12 different ratios. Three four-day pe-

riods were devoted to each ratio of calcium to phosphorus intake. The first five periods concerned preliminary observations without vitamin D, but after that 1 cc. of Vigantol, an oily solution of irradiated ergosterol containing 15,000 international units of vitamin D per cc., was given daily.

Subject 2, L. Y. H., was a man of 24 with syphilitic osteitis of right radius and tibia, the rest of the skeleton showing normal density and texture on x-ray examination. As localized bone involvement by infection usually does not give rise to general metabolic disturbances, the patient may be regarded as a control for the present purpose. He was given Diets 1, 2 and 3 in that sequence. With each diet, namely, with each level of phos-

TABLE I  
*Composition of diets in grams per day†*

Articles of food	Vitamin D	Subject 1 (H. F. M.)						Subject 2 (L. Y. H.)		
		Diet 1	Diet 2	Diet 3	Diet 4	Diet 5a	Diet 5b	Diet 1	Diet 2	Diet 3
Millet.....	±	50		50	50					100
Rice.....	±		50						200	100
Glutinous rice.....						20	16		50	
Oatmeal.....	±				100					
White wheat flour.....	+	50	150	200	75	150	120	300	200	150
Mung bean flour.....						100	80			
Peanut.....	*				25					
Egg.....	+			30	30					
Egg white.....	±					300	240	300	100	
Pork.....	*	150	75	50	25				25	100
Chicken.....	*	100	50	50	75					100
Beef.....	±		50	75	75					
Aroid.....	*	50	50	50						100
Potato.....	*	50							50	50
Sweet potato.....	±				50					
Carrot.....	±				50					
Turnip.....	±		50	100		100	80			
Cabbage.....	±	100	100	150	100					100
Onion.....	*								50	
Chinese lettuce.....									100	
Spinach.....	±							100		
Apple.....	*	50	50	50						50
Banana.....	*					50	40			
Orange.....	*					50	40			
Lard.....								50	31	
Butter.....	++							30	20	
Sesame oil.....		32	45	50	50	70	56	40	20	50
Table salt.....		4	4	4	4	4	3.2	4	6	6
Sugar.....		21	20	20	24	50	40	60	24	
Soy bean sauce.....		5	5	5	5	5	4			
Protein.....		65	59	69	77	58	46	71	62	80
Carbohydrate.....		102	192	232	211	284	227	286	392	307
Fat.....		63	70	79	94	73	58	120	76	76
Calories.....		1235	1642	1923	2000	2022	1614	2508	2500	2232
Calcium.....		0.138	0.140	0.191	0.181	0.176	0.140	0.178	0.118	0.173
Phosphorus.....		0.914	0.627	0.925	1.163	0.324	0.259	0.402	0.582	1.094
Nitrogen.....		10.40	9.12	10.68	12.39	8.28	6.64	10.82	8.69	11.51

† Calcium, phosphorus and nitrogen values are actually determined, and vitamin D values taken from Wu (8); ++ good amount; + fair amount; ± no appreciable amount; \* doubtful or undetermined. Approximate computation of the acid base balance of the diets according to Sherman (9) gives potential acidities ranging from 19 to 35 cc. of normal acid for Subject 1, and 30 to 44 cc. for Subject 2.

phorus intake, calcium was raised by the addition of desired amounts of calcium lactate. With this patient, 3 levels each of calcium and phosphorus were studied, giving 9 combinations. No vitamin D was administered.

Calculation of the acid base balance of the diets according to Sherman (9) showed that all the diets were potentially acid with relatively small variations, but the computation should be considered only approximate, because of the uncertainty of the applicability of Sherman's figures to local foodstuffs.

Stool and urine respectively of each period were pooled for analysis. Venepuncture was done before breakfast at the beginning of each period. Metabolic ward routine and analytical methods for calcium, phosphorus and nitrogen of food, urine, stool and blood were described previously (7).

## RESULTS

*Serum calcium and phosphorus.* As seen from Figure 1, Subject 1 maintained a fairly stationary level of serum calcium throughout the period of 212 days of continuous observation, the range be-

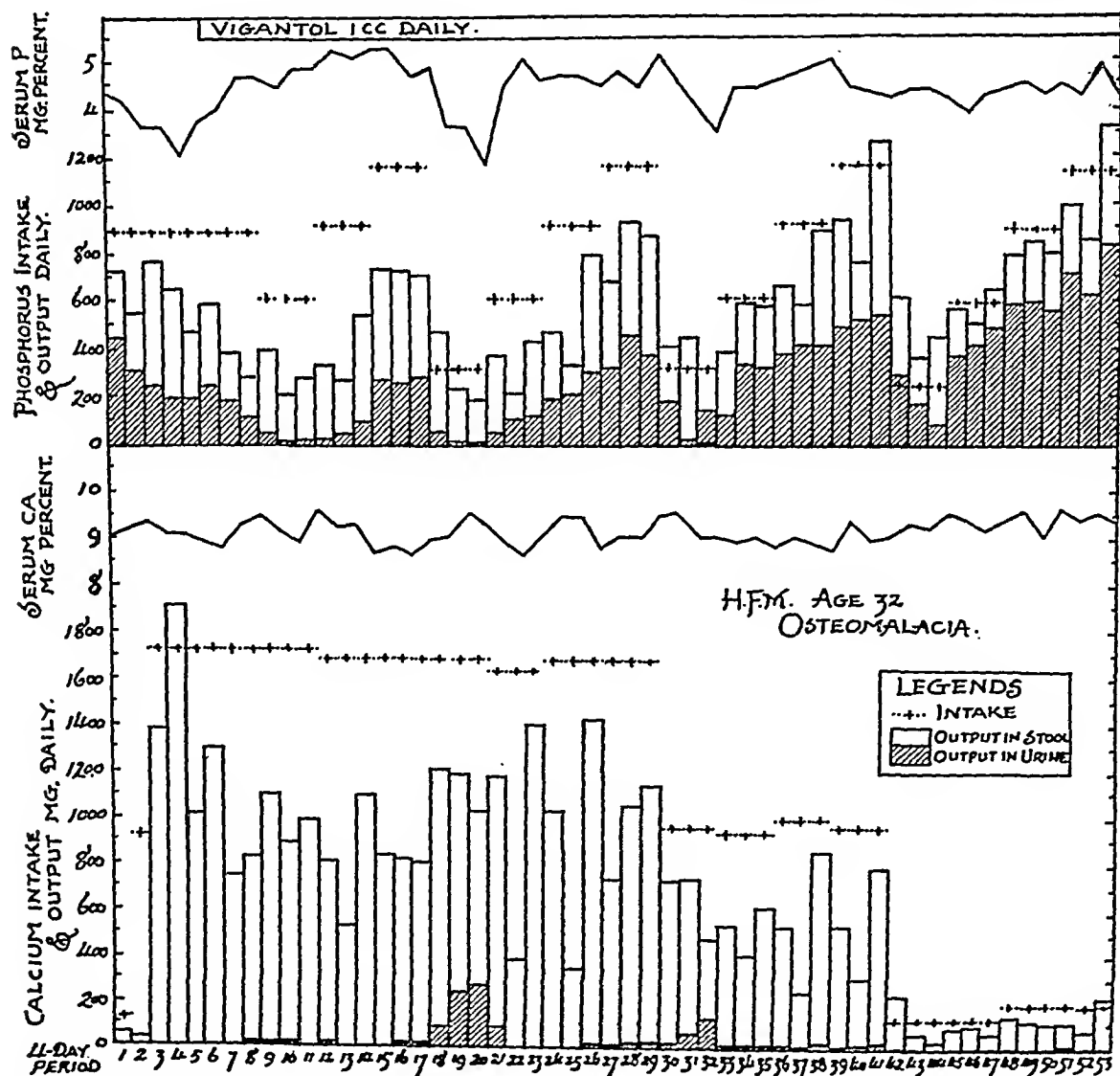


FIG. 1. CALCIUM AND PHOSPHORUS METABOLISM AND THEIR SERUM LEVELS IN RELATION TO VARYING INTAKE OF CALCIUM AND PHOSPHORUS IN SUBJECT 1

ing from 8.7 to 9.6 mgm. per 100 cc. and the trend bearing no apparent relation to the dietary changes. The serum inorganic phosphorus level, however, varied from 2.9 to 5.3, a difference of 2.4 mgm. per 100 cc. The phosphorus level, beginning at 4.3 mgm. per 100 cc., gradually went down to 3.0 as calcium intake in the diet was stepped up to 1.7 grams (Period 4). While the calcium intake was maintained at this high level, the phosphorus curve began to climb as vitamin D was given, and rose to a maximum of 5.3 as the phosphorus intake was progressively raised (Period 15). The phosphorus curve showed a second drop in Periods 18 to 20 when the phosphorus intake was suddenly decreased to a minimum, and a subsequent recovery to the high level in Periods 27 to 29 when high phosphorus intake was restored. When calcium intake was maintained at a lower level, namely, 1.0 gram as in Periods 30 to 41, similar changes in the phosphorus intake brought about a repetition of the cycle of events in the serum phosphorus curve, but to a lesser extent. But when the calcium intake was kept minimal (Periods 42 to 53), lowering of the phosphorus intake failed to elicit any significant change in serum phosphorus. In other words, serum phosphorus varied more with the ratio of calcium to phosphorus than with their actual levels in the intake. Whenever the ratio is high serum phosphorus drops.

In Subject 2 (Figure 2) the serum calcium level was also relatively constant, varying from a minimum of 9.0 to a maximum of 10.2 mgm. per 100 cc., irrespective of the calcium and phosphorus intake. The serum phosphorus, compared with that of Subject 1, showed much less fluctuation, ranging as it did between 3.9 and 5.1 mgm. per 100 cc. Moreover, the trend of variation with dietary intake seemed to be opposite in direction to that seen in Case 1. When the calcium supply was short in relation to phosphorus (low Ca:P ratio), the serum phosphorus tended to fall with a subsequent rise when calcium intake was stepwise increased. However, the changes observed were not sufficiently pronounced to render their significance indubitable.

From the above observations it may be concluded that dietary variations of calcium and phosphorus are not significantly reflected in the serum calcium level. This is true in osteomalacia, as

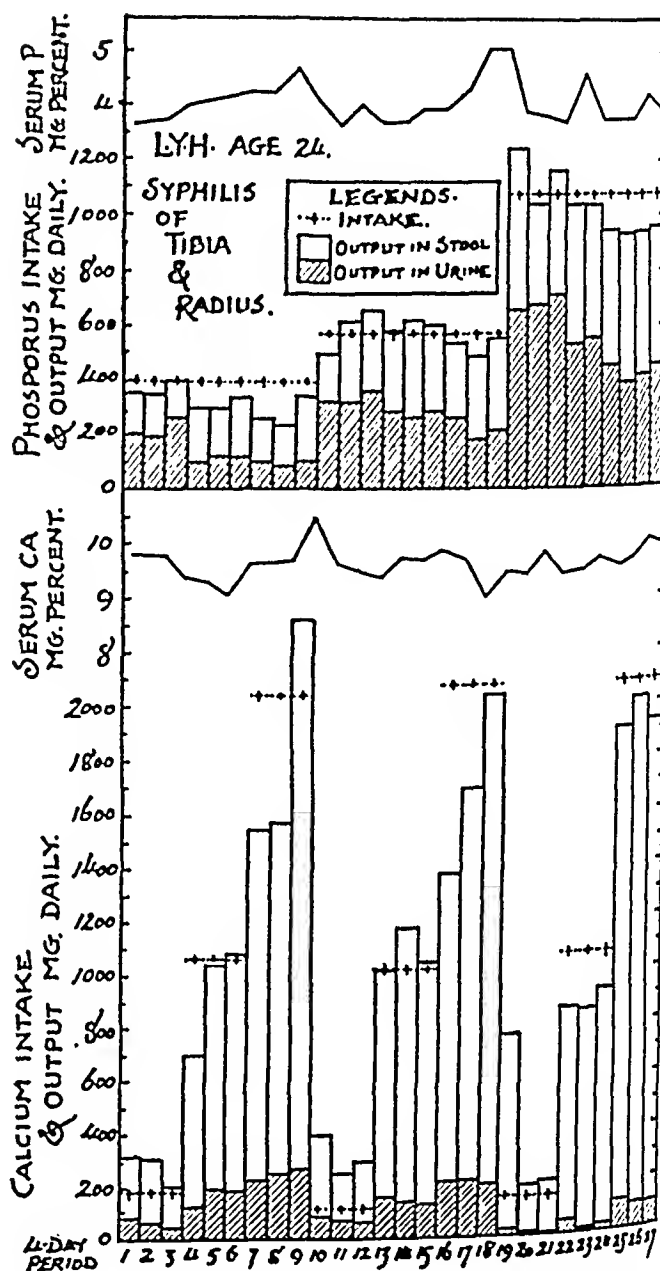


FIG. 2. CALCIUM AND PHOSPHORUS METABOLISM AND THEIR SERUM LEVELS IN RELATION TO VARYING INTAKE OF CALCIUM AND PHOSPHORUS IN SUBJECT 2

well as in the control case, probably on account of the protective influence of vitamin D, as in its absence the serum calcium and inorganic phosphorus reflect remarkably the ratio of these elements in the diet as shown by Shohl (10) in experimental rickets in rats. Dietary changes may cause fluctuations in serum phosphorus, however, even when vitamin D is added to the diet. In healing osteomalacia, for example, where there is marked deposition of calcium and phosphorus in the bones, a deficiency in intake of phosphorus relative to calcium intake results in a fall in serum phosphorus; and excess phosphorus intake rela-

tive to calcium intake may result in a rise in serum phosphorus. On the other hand, in the case of localized bone disease and presumably in normal individuals where excesses in supply are excreted and deficiencies in intake are made up from the large skeletal store, serum phosphorus level is less subject to fluctuation.

*Paths of excretion.* The data from Subject 1 as presented in Figure 1 and as averaged in Table II demonstrate a general reciprocal relationship between urinary calcium and phosphorus. At a constant level of calcium intake, progressive increment of phosphorus supply tended to decrease the urinary calcium sometimes to the point of disappearance, and at the same time to augment the urinary phosphorus.

If the results at the same level of dietary phosphorus are taken for comparison successive addition of calcium intake increased the urinary calcium coincident with a gradual and steady diminution of urinary phosphorus. In general, the magnitude of urinary excretion of calcium was small or negligible but it became considerable when calcium was supplied far in excess of the dietary phosphorus (Periods 18 to 20). Likewise, urinary excretion of phosphorus was very much limited on low phosphorus diet, but became dominant

when phosphorus supply was far in excess of dietary calcium (Periods 51 to 53).

Fecal calcium increased consistently with the calcium intake, having little relation with the phosphorus supply, while fecal phosphorus was directly related not only with dietary phosphorus, but also with dietary calcium. Intestinal elimination of phosphorus, then, depends not only on its supply in the diet, but also on the amount of calcium presented in the intestine for excretion.

In Subject 2 (Figure 2 and Table III) similar results were obtained. The magnitude of urinary calcium excretion was greater, and it never disappeared, even when the supply was minimal in the presence of large phosphorus intake (Periods 19 to 21). Likewise, when phosphorus intake was minimal in the presence of excessive dietary calcium, urinary phosphorus, though decreased, was still considerable, compared with that in healing osteomalacia. The fecal elimination of calcium and, to a lesser degree, of phosphorus in Subject 2 was greater also, but the correlation between the intake of calcium and phosphorus and their output in the stool was just as close. In addition to the tendency for fecal phosphorus to vary directly with calcium intake, there was a

TABLE II  
Subject 1. Average daily calcium and phosphorus metabolism

Period number	Diet number	Intake			Output					Balances				
		Ca	P	Ratio Ca : P	Urinary		Fecal			Ca	P	N <sub>2</sub>	P corrected	Ratio Ca : P corrected
					Ca	P	Ca	P	Dry weight					
		mgm.	mgm.		mgm.	mgm.	mgm.	mgm.	grams	mgm.	mgm.	grams	mgm.	
3-5	1	1738	914	1.90	0	222	1444	420	19.4	294	272	1.05	212	1.39
*6-8	1	1738	914	1.90	5	190	956	238	12.7	777	486	1.43	404	1.93
9-11	2	1740	627	2.14	19	25	962	283	17.0	759	319	1.22	249	3.05
12-14	3	1691	925	1.83	7	62	822	328	17.9	862	535	2.24	406	2.12
15-17	4	1681	1163	1.45	6	297	829	438	22.8	846	428	2.40	287	2.94
18-20	5	1676	324	5.18	209	23	945	287	20.1	522	14	0.98	-44	
21-23	2	1640	627	2.60	31	85	970	261	18.9	639	281	0.71	239	2.67
24-26	3	1691	925	1.83	4	238	935	296	18.3	752	391	1.56	301	2.50
27-29	4	1681	1163	1.45	19	393	960	444	22.2	702	326	2.17	201	3.49
30-32	5	976	324	3.01	77	37	582	318	19.7	313	-31	1.59	-124	
33-35	2	940	627	1.50	6	252	545	249	15.9	389	126	0.22	113	3.44
36-38	3	994	924	1.08	8	413	535	301	16.5	451	210	2.17	82	5.50
39-41	4	981	1163	0.84	6	528	540	408	19.0	435	227	1.50	139	3.13
42-44	5a	141	259	0.54	3	184	111	299	18.4	27	-224	-0.01	-223	
45-47	2	140	627	0.22	4	436	78	157	11.6	58	34	0.70	-6	
48-50	3	194	924	0.21	1	593	129	242	18.3	64	89	1.40	7	
51-53	4	181	1163	0.16	4	744	137	335	20.2	40	84	1.11	20	2.00

\* Vigantol 1 cc. daily started from this period and continued throughout.

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In Subject 2 (Figure 2) the serum calcium level was also relatively constant, varying from a minimum of 9.0 to a maximum of 10.2 mgm. per 100 cc., irrespective of the calcium and phosphorus intake. The serum phosphorus, compared with that of Subject 1, showed much less fluctuation, ranging as it did between 3.9 and 5.1 mgm. per 100 cc. Moreover, the trend of variation with dietary intake seemed to be opposite in direction to that seen in Case 1. When the calcium supply was short in relation to phosphorus (low Ca:P ratio), the serum phosphorus tended to fall with a subsequent rise when calcium intake was stepwise increased. However, the changes observed were not sufficiently pronounced to render their significance indubitable.

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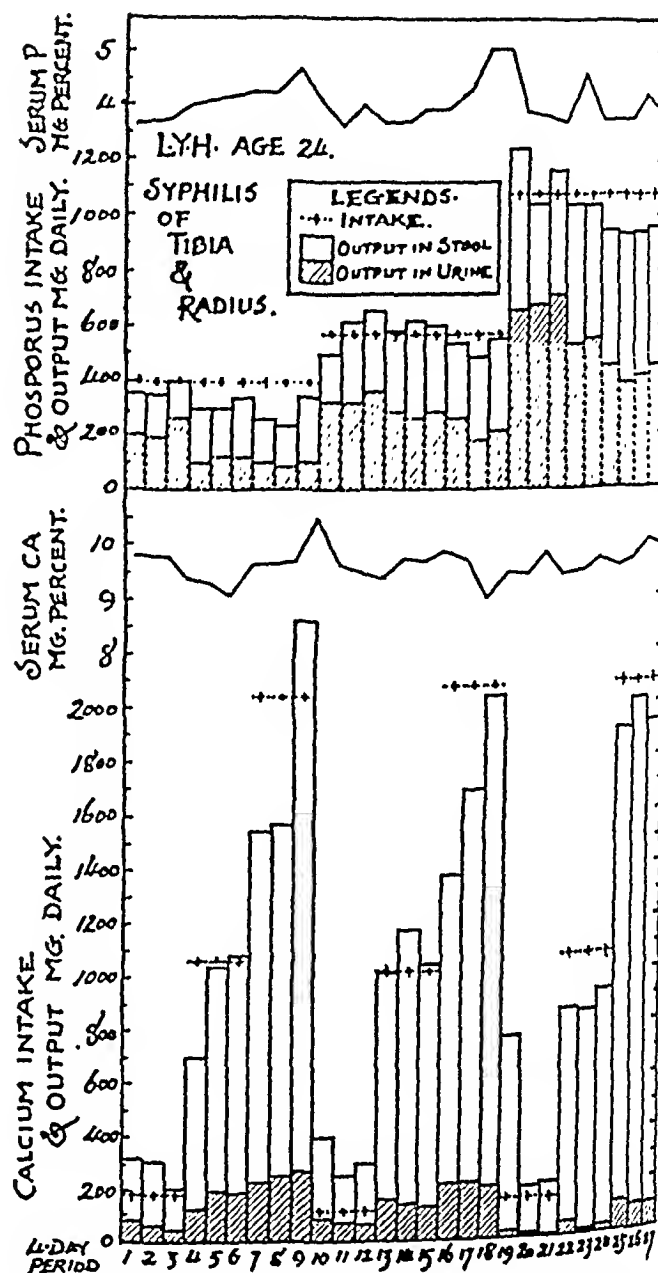


FIG. 2. CALCIUM AND PHOSPHORUS METABOLISM AND THEIR SERUM LEVELS IN RELATION TO VARYING INTAKE OF CALCIUM AND PHOSPHORUS IN SUBJECT 2

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In Subject 2 (Figure 2 and Table III) similar results were obtained. The magnitude of urinary calcium excretion was greater, and it never disappeared, even when the supply was minimal in the presence of large phosphorus intake (Periods 19 to 21). Likewise, when phosphorus intake was minimal in the presence of excessive dietary calcium, urinary phosphorus, though decreased, was still considerable, compared with that in healing osteomalacia. The fecal elimination of calcium and, to a lesser degree, of phosphorus in Subject 2 was greater also, but the correlation between the intake of calcium and phosphorus and their output in the stool was just as close. In addition to the tendency for fecal phosphorus to vary directly with calcium intake, there was a

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					Ca	P	Ca	P	Dry weight					
		mgm.	mgm.		mgm.	mgm.	mgm.	mgm.	grams	mgm.	mgm.	grams	mgm.	
3-5	1	1738	914	1.90	0	222	1444	420	19.4	294	272	1.05	212	1.39
*6-8	1	1738	914	1.90	5	190	956	238	12.7	777	486	1.43	404	1.93
9-11	2	1740	627	2.14	19	25	962	283	17.0	759	319	1.22	249	3.05
12-14	3	1691	925	1.83	7	62	822	328	17.9	862	535	2.24	406	2.12
15-17	4	1681	1163	1.45	6	297	829	438	22.8	846	428	2.40	287	2.94
18-20	5	1676	324	5.18	209	23	945	287	20.1	522	14	0.98	-44	
21-23	2	1640	627	2.60	31	85	970	261	18.9	639	281	0.71	239	2.67
24-26	3	1691	925	1.83	4	238	935	296	18.3	752	391	1.56	301	2.50
27-29	4	1681	1163	1.45	19	393	960	444	22.2	702	326	2.17	201	3.49
30-32	5	976	324	3.01	77	37	582	318	19.7	313	-31	1.59	-124	
33-35	2	940	627	1.50	6	252	545	249	15.9	389	126	0.22	113	3.44
36-38	3	994	924	1.08	8	413	535	301	16.5	451	210	2.17	82	5.50
39-41	4	981	1163	0.84	6	528	540	408	19.0	435	227	1.50	139	3.13
42-44	5a	141	259	0.54	3	184	111	299	18.4	27	-224	-0.01	-223	
45-47	2	140	627	0.22	4	436	78	157	11.6	58	34	0.70	-6	
48-50	3	194	924	0.21	1	593	129	242	18.3	64	89	1.40	7	
51-53	4	181	1163	0.16	4	744	137	335	20.2	40	84	1.11	20	2.00

\* Vigantol 1 cc. daily started from this period and continued throughout.



TABLE III  
Subject 2. Average daily calcium and phosphorus metabolism

Period number	Diet number	Intake			Output					Balances			
		Ca	P	Ratio Ca : P	Urinary		Fecal			Ca	P	N <sub>2</sub>	P corrected
					Ca	P	Ca	P	Dry weight				
		mgm.	mgm.		mgm.	mgm.	mgm.	mgm.	grams	mgm.	mgm.	mgm.	mgm.
1-3	1	178	402	0.44	73	232	207	153	20.3	-102	28	1.65	-69
4-6	1	1058	402	2.63	189	158	736	155	20.5	133	89	2.27	-45
7-9	1	2043	402	5.08	252	104	1561	183	18.2	230	115	2.33	-22
10-12	2	118	582	0.20	79	341	241	255	16.5	-202	-14	1.16	-82
13-15	2	1023	582	1.76	153	282	928	323	21.4	58	-23	1.09	-87
16-18	2	2080	582	3.58	211	221	1495	319	21.2	374	42	1.16	-26
19-21	3	173	1094	0.16	19	695	373	471	21.0	-219	-72	0.60	-107
22-24	3	1078	1094	0.99	36	519	859	492	20.7	183	83	0.50	54
25-27	3	2084	1094	1.91	116	414	1812	525	25.4	156	155	0.48	127

discernible but slight tendency for the fecal calcium to vary directly with phosphorus intake.

As the average daily dry fecal weights in both cases varied only slightly on the various diets, it is unlikely that variations in roughage were sufficiently large to play an important rôle in the intestinal elimination of calcium and phosphorus.

**Balances.** While calcium balance may be taken to represent the state of bone metabolism, phosphorus balance is under the dual influence of bone and soft tissue metabolism. For every 17 grams of nitrogen retained or lost, 1 gram of phosphorus is retained or lost. To calculate the amount of phosphorus actually involved with calcium in bone metabolism, the total phosphorus balance is corrected by an amount equivalent to nitrogen balance. The corrected phosphorus balances are set forth in Tables II and III. In Table II it may be of interest to note the remarkable effect of vitamin D on calcium balance in osteomalacia. Prior to vitamin D administration the patient retained 294 mgm. of calcium on an intake of 1738 mgm. per day (Periods 3 to 5), but after its administration the retention increased to 777 mgm. on the same intake (Periods 6 to 8), the improvement being mainly due to lessened elimination in the stool.

To facilitate comparison, Tables IV and V are constructed in which calcium and corrected phosphorus balances are grouped according to intake. Subject 1 (as seen in the upper part of Table IV) exhibited a striking dependence of calcium balance on calcium intake. Calcium retention on the aver-

age increased steadily from 47 to 654 mgm. per day as calcium intake was progressively raised from 164 to 1672 mgm. per day, regardless of phosphorus intake. On the other hand, at a given

TABLE IV  
Subject 1. The effect of calcium and phosphorus intake on their balances

Calcium intake		Calcium balance at the phosphorus intake of				Average Ca balance at same Ca intake regardless of P intake
Range	Level	324 mgm.	627 mgm.	922 mgm.	1163 mgm.	
mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
140-194	164	0.52* 27	0.22 58	0.21 64	0.16 40	47
940-994	973	3.01 313	1.60 389	1.08 451	0.84 435	397
1640-1691	1672	5.18 522	2.60 639	1.88 752	1.45 702	654
Average of Ca balance at same P intake regardless of Ca intake		287	362	422	392	
Calcium intake		Phosphorus balance at the phosphorus intake of				Average P balance at same Ca intake regardless of P intake
Range	Level	324 mgm.	627 mgm.	922 mgm.	1163 mgm.	
140-194	164	0.52 -223	0.22 -6	0.21 7	0.16 20	-50
940-994	973	3.01 -124	1.60 113	1.08 82	0.84 139	52
1640-1691	1672	5.18 -44	2.60 239	1.88 301	1.45 201	174
Average P balance at same P intake regardless of Ca intake		-130	115	130	120	

\* Figures in italics are ratios of calcium to phosphorus intake.

level of calcium intake, progressive increment of dietary phosphorus up to 922 mgm. per day resulted in a slight ascending tendency in the calcium balance, but further increase to 1163 mgm. failed to improve the calcium balance which, in fact, fell somewhat at the latter level of phosphorus intake. Corrected phosphorus balance likewise depended more on calcium than on phosphorus intake. When calcium intake was raised from 164 to 1672 mgm., the average phosphorus balance increased from -50 to +174 mgm.; whereas various levels of phosphorus intake made no striking difference to the phosphorus balance except in the case of minimal phosphorus intake where negative balance prevailed.

Balance data on Subject 2, summarized in Table V, show essentially the same findings, namely, the greater importance of calcium as the limiting factor in both calcium and phosphorus balances. However, on a minimal calcium intake of 145 mgm. he lost on the average 174 mgm. per day in

contrast to Subject 1 who gained 47 mgm. on an intake of 164 mgm. The degree of calcium loss on a minimal intake in Subject 2 was within normal limits (11), while the behavior of Subject 1 was usually conservative. Thus the latter stored approximately 95 grams of calcium and 43 grams of phosphorus in the period of 200 days, equivalent to 15 per cent of the stores which should be in the body, in contrast to the control patient who retained only 5.9 grams of calcium and 4.8 grams of phosphorus in 108 days.

Examination of the ratios of calcium to corrected phosphorus balance (Table II) shows that they are above 2 in the majority of instances, and above 3 in several instances, bearing no close relationship with the ratios of intake. If we accept the mineral composition of normal bone as  $\text{CaCO}_3 \cdot 2\text{Ca}_3(\text{PO}_4)_2$  according to the x-ray analysis of Roseberry, Hastings and Morse (12), then the ratio of Ca:P should be 2.26. The fact that the ratios of retention in Subject 1 were usually higher than that prescribed for normal bone would suggest that more calcium was deposited as  $\text{CaCO}_3$  than  $\text{Ca}_3(\text{PO}_4)_2$  in the new bone formation or that calcium suffered to a greater extent than phosphorus during the prior demineralization. As to the actual amount of calcium retained, the maximum was 752 mgm. (or 45 per cent of intake) on an intake of 1672 mgm. calcium and 922 mgm. phosphorus giving a ratio of 1.83 (Table IV). This happens to be also the level and ratio of intake associated with the largest retention of phosphorus, namely, 301 mgm. (or 32 per cent of intake). Thus both calcium and phosphorus have to be given at fairly high levels with a ratio approaching 2 in order to secure maximal retention of both elements. Otherwise, the ratio made very little difference to the calcium balance which depended mainly on the level of intake, in conformity with the work of Shohl (10).

TABLE V

Subject 2. The effect of calcium and phosphorus intake on their balances

Calcium intake		Calcium balance at the phosphorus intake of			Average Ca balance at same Ca intake regardless of P intake
Range	Level	402 mgm.	582 mgm.	1094 mgm.	
		mgm.	mgm.	mgm.	mgm.
112-178	145	<i>0.44</i> <sup>*</sup> -102	<i>0.20</i> -202	<i>0.16</i> -219	-174
1012-1078	1040	<i>2.63</i> 113	<i>1.76</i> -58	<i>0.39</i> 183	86
2012-2084	2055	<i>6.08</i> 230	<i>3.68</i> 374	<i>1.91</i> 156	253
Average Ca balance at same P intake regardless of Ca intake		87	38	40	
Calcium intake		Phosphorus balance at the phosphorus intake of			Average P balance at same Ca intake regardless of P intake
Range	Level	402 mgm.	582 mgm.	1094 mgm.	
112-178	145	<i>0.44</i> -69	<i>0.20</i> -82	<i>0.16</i> -107	-86
1012-1078	1040	<i>2.63</i> -45	<i>1.76</i> -87	<i>0.39</i> 54	-26
2012-2084	2055	<i>6.08</i> -22	<i>3.68</i> -26	<i>1.91</i> 126	26
Average P balance at same P intake regardless of Ca intake		-45	-65	24	

\* Figures in italics are ratios of calcium to phosphorus intake.

## DISCUSSION

The present results obtained with the patient with osteomalacia receiving continuous administration of vitamin D are in entire agreement with those of previous studies on patients whose treatment with vitamin D was discontinued after relatively short periods of 4 to 6 weeks when its maximum effect had been attained. The behavior of

serum calcium and phosphorus, the manner of conservation of these elements through the urinary and intestinal tracts, the ability of the patients to maintain positive calcium balance on minimal intake and to retain large amounts of it on higher levels of intake, and finally the relatively greater importance of calcium intake rather than phosphorus intake as the limiting factor in both calcium and phosphorus retention are essentially the same in both instances. Thus vitamin D, once given to the extent of its maximum effect, will maintain its action unabated for at least several months after its discontinuation, and its continuous administration in the treatment of osteomalacia does not seem to offer any substantial advantage.

In the therapy of osteomalacia, while vitamin D administration corrects the basic metabolic defect, it is essential that both calcium and phosphorus be given at fairly high levels, preferably with a ratio of approximately 2 in order to promote large retention of both elements and therefore rapid restoration of the mineral contents of the depleted osseous system. With ordinary Chinese dietaries which are low in calcium and fairly high in phosphorus, the desired high level of calcium intake has to be supplied as calcium salts, while that of phosphorus intake can easily be taken care of by the diet.

The behavior of the patient without skeletal decalcification resembles that of patients having healing osteomalacia in respect to the reciprocal relationship between urinary calcium and phosphorus, the approximately parallel relationship between stool calcium and phosphorus, the dependence of both calcium and phosphorus balances on calcium intake, and the slight effect of phosphorus intake as a limiting factor in both calcium and phosphorus retention. On the other hand, he differs in that the serum phosphorus is relatively more stable toward dietary changes, and in that the magnitudes of urinary and stool excretion of calcium and phosphorus are greater, resulting in negative balance on low intake and only slight retention on higher levels of intake. These differences are but an expression of the fact that in a relatively normal individual measures of mineral conservation are less urgently in need of ap-

n.

## SUMMARY

1. In two patients, one with osteomalacia under reparation and the other with syphilitic osteitis of radius and tibia, the serum levels of calcium and phosphorus, paths of excretion and retention of these elements were studied in relation to their levels and ratios of intake.

2. Serum calcium was fairly constant in both cases, while serum inorganic phosphorus tended to lower with a higher ratio of Ca:P intake in the first patient. No such variations were demonstrable in the second patient.

3. A reciprocal relationship between calcium and phosphorus in urine was shown in both instances. Whenever the Ca:P ratio in the diet was high, urinary calcium increased, while urinary phosphorus diminished. An opposite change in the ratio resulted in a diminution of urinary calcium coinciding with a rise of urinary phosphorus.

4. Fecal calcium and phosphorus varied with their respective level of intake, while fecal phosphorus was also partly dependent on calcium intake.

5. Retention of either calcium or phosphorus depended more on the calcium than phosphorus intake, although both had to be supplied in fairly large quantities with a Ca:P ratio of approximately 2 in order to realize maximal retention of both elements to promote efficient repair of skeletal demineralization in osteomalacia.

6. The present study with prolonged administration of vitamin D revealed no essential difference from the previous work with limited vitamin D therapy, showing that the effect of vitamin D lasts long after its discontinuation.

## CASE HISTORIES

*Case 1.* H. F. M., a Chinese housewife of 32, was admitted on August 28, 1935 for pain in back and legs and difficulty in walking. These began 7 years prior to admission when she had her first pregnancy. Labor was spontaneous but lasted for more than 24 hours. After that she had periodical exacerbations of the above symptoms in winter and spring when she kept herself indoors. The second pregnancy occurred 4 years after the first and resulted in a seven months' premature labor lasting more than 48 hours. Henceforth symptoms became worse. She could neither stand on her feet nor walk without support. Her diet had always been extremely poor. In the cold months she lived on cereals and salted vegetables. In the warmer months some fresh vegetables

were available. Meat was seldom taken and eggs only occasionally.

Examination on admission confirmed her statement about her inability to stand or walk without support. Body weight was 40 kgm. and height 136 cm. On lying down, the right thigh was slightly flexed and abducted, with the knee joint held in 30° flexion. Movement at the hip joint caused pain. There was no tenderness along the lower extremities. The pelvis was of the funnel type. Symphysis pubis protruded and sacrum was prominent. Tenderness was marked over the sacro-iliac joints, pelvic bones, lower ribs and lumbar vertebrae. The right upper molar teeth and lower incisors were loose. Other physical findings were normal. X-ray showed a deformed pelvis and general osteoporosis and some pleural thickening with adhesions in the right lower chest. Blood calcium was 8.92, phosphorus 3.29 mgm. per cent, and plasma phosphatase 4.9 units (Bodansky). Blood counts and urinalysis were essentially normal. Stool was positive for ova of ascaris. Metabolic studies were carried out in four-day periods for 212 days from October 4, 1935 to May 2, 1936. Besides the dietary treatment and vitamin D administration she also received physiotherapy in the form of exercises for extension of the hip and spine and infra-red irradiation. At the time of discharge she could walk fairly well without support, and x-ray of bones revealed definitely increased density.

*Case 2.* L. Y. S., a Chinese man of 24, entered on July 13, 1934 for swelling and lengthening of the right leg of 8 years' duration and of the right forearm of two years' duration. Onset was insidious without history of injury. He had occasional low grade fever and pain after prolonged walking. He had had venereal exposures.

The patient was found to be slightly undernourished. Body weight was 42.2 kgm. and height 159 cm. His right leg was 5.5 cm. longer than the left and also bigger especially in the lower part. There was slight tenderness over the right tibia, and the overlying skin was slightly warmer than that of the left side. The knee and ankle were not involved. Right forearm was 1.5 cm. longer than the left. The elbow and wrist were free. Other physical findings were essentially normal. X-ray of the bones showed irregular areas of condensation and rarefaction in the cortex involving the entire length of right tibia and radius. The rest of the skeleton appeared normal. Blood and urine examinations revealed no significant findings. Stools contained ova of ascaris. Blood calcium was 9.7 and phosphorus 4.2 mgm. per cent. Basal metabolic rate was +8.4 per cent. Blood Wassermann

and Kahn tests were strongly positive. Metabolic studies were carried on for 108 days (27 four-day periods from September 18, 1934 to January 3, 1935). Subsequent intensive antisyphilitic treatment to date has given rise to marked improvement in the bone lesions.

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# STUDIES IN IRON TRANSPORTATION AND METABOLISM. I. CHEMICAL METHODS AND NORMAL VALUES FOR PLASMA IRON AND "EASILY SPLIT-OFF" BLOOD IRON

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Iron is now recognized in three forms in the blood: hemoglobin iron, plasma iron, and "easily split-off" iron. The last has been so termed because weak acids readily free it from its lightly bound state in the erythrocytes and in plasma. Barkan (1), Lintzel (2), Starkenstein and Weden (3), Dominici (4), and others (5, 11) have gone far toward defining this "leicht abspaltbare Bluteisen" fraction, and Barkan has demonstrated that it may be subdivided into two parts: E, comprising about 60 to 70 per cent of the whole and present only in the erythrocytes, is bound by carbon monoxide in such a manner that it is protected against the "splitting-off" action of weak acids; and E', constituting about 30 to 40 per cent of the fraction and present in plasma as well as in the red cells, is not bound by carbon monoxide. In addition to these three forms, iron is also present in the nuclei of the leukocytes in minute amounts comparable to those found by Warburg in practically all cells of the body as a part of the respiratory enzyme mechanism.

Plasma iron and "easily split-off" iron have been the subject of many recent investigations in an attempt to determine their physiological significance. Progress in this direction has been delayed primarily because the small amounts of iron present in these two fractions are difficult to determine. Most of the earlier techniques lack accuracy and dependability. The amount of hemoglobin iron is so many times greater than the amounts of non-hemoglobin iron in blood that it is difficult sharply to separate the one from the other. Normal values reported by different investigators for plasma and for "easily split-off" blood iron have shown wide variations. Consequently, a skeptical attitude, not entirely unjusti-

fied, has arisen concerning the very existence and independent identity of the non-hemoglobin iron fractions.

The present series of studies has been directed toward a better understanding of the mechanism and significance of iron transportation and utilization. A knowledge of the normal zonal range for the plasma and "easily split-off" blood iron fractions with an accurate measure of the dependability of the methods used in determining them is fundamental. This communication, therefore, will attempt to analyze critically the methods of quantitation that have been used in the past; will present certain changes in technique which have been found of help in establishing the dependability of the chemical analyses; and from original data accumulated in this laboratory, and from the literature, will suggest the limits for the range of normal variation.

## A. PLASMA AND SERUM IRON

### *I. The non-hemoglobinous nature of plasma iron*

Because it has been extremely difficult to measure quantitatively the minute amounts of hemoglobin due to hemolysis which may be present in blood plasma or serum, there has been considerable hesitancy about accepting the small amounts of iron found in plasma as really of non-hemoglobinous origin. Whole human blood contains approximately 50 mgm. per cent hemoglobin iron, a quantity of iron 500 times greater than the amounts reported by most observers as occurring in normal plasma (range 50 to 180 micrograms per cent). The erythrocytes from which the plasma or serum samples are separated must necessarily be subjected to the trauma which always accompanies venesection and centrifugation so that some hemolysis is almost unavoidable; and only 18 to 51 mgm. per cent hemoglobin needs be present in plasma to account for all the iron nor-

<sup>1</sup> These studies were begun during the tenure of a National Research Council Fellowship in the Department of Medical and Surgical Research, Ohio State University.

mally found. It will be apparent, however, that abundant experimental evidence exists upon which to establish plasma iron as a non-hemoglobinous form of iron.

Henriques and Roche (6), Marlow and Taylor (7), and others examined plasma and serum samples spectroscopically for the absorption bands of oxyhemoglobin before making their determinations. Henriques and Roche (6) found spectroscopic methods capable of detecting 3 to 5 mgm. per cent hemoglobin (10 to 16.5 micrograms per cent hemoglobin iron). By adding to their sera a known amount of hemoglobin and rediluting with graduated dilutions until the absorption bands of oxyhemoglobin just faded out, they were able to quantitate the amount of hemoglobin present within a stated accuracy of 10 per cent and to prove that only a fraction of the iron present in the samples analyzed by them was hemoglobin iron.

Fowweather (8), by drawing blood very carefully into an oiled syringe, transferring it promptly to a paraffined tube and centrifuging immediately at high speed, was able to throw the blood platelets down with the other formed elements of blood before coagulation began. The plasma thus obtained contained no anticoagulant and gave a negative benzidine reaction with a benzidine reagent whose sensitivity was such that it could detect one part per million of hemoglobin (less than 1 microgram per cent hemoglobin iron). Bing and Hanzal (9), working with dog blood, were unable to duplicate Fowweather's results and again considered it necessary to find some way of measuring the amounts of hemoglobin present in the plasma or serum with which they were working. They devised a colorimetric method based on the color formed with benzidine; but because of the proteins present in plasma and serum, only about 75 per cent of a known amount of added hemoglobin could be recovered. Hence, on each set of determinations a factor of recovery had to be determined and all results corrected accordingly.

By slightly modifying Fowweather's method, we have been able to obtain with fair consistency human sera,<sup>2</sup> which contained less than 1 micro-

gram per cent of hemoglobin iron. Instead of using an oiled syringe, blood was permitted to flow from an 18 gauge needle directly into a paraffined tube, immediately following which the blood was centrifuged at 2500 r.p.m. for 45 minutes. Occasionally, slight hemolysis occurred, particularly when important blood specimens had to be collected at some distance from the laboratory and immediate centrifugation was impossible. Under such circumstances, a quantitative determination of the hemoglobin iron present in the serum became necessary. The following method, based on the benzidine-hemoglobin color reaction, was accordingly devised.

Crystalline benzidine dihydrochloride was prepared according to the method of Leiboff (10). One cc. of a freshly prepared 1 per cent aqueous solution of this reagent was placed in a glass comparator tube (vial 11 × 2 cm.) along with varying quantities of dilute hemoglobin solutions of known concentration and the volume made up to 10 cc. with distilled water. To this was added 1 cc. of 3 per cent hydrogen peroxide, and the whole observed for the development of the characteristic blue color reaction. Two blanks for comparison were similarly prepared, one with the hemoglobin solution omitted, and the other with no hydrogen peroxide added. Since the color gradually developed to its maximum intensity and then immediately began to fade, comparison in a colorimeter was not practical. The comparator tubes, instead, were observed through the depth of the fluid against a white background, daylight being used as the source of light, and the smallest amount of hemoglobin (0.2 to 0.4 microgram under these circumstances) which would just develop a perceptible color with the benzidine reagent was determined. In a similar manner, the smallest quantity of any particular serum or plasma preparation which would just give a perceptible color with the benzidine was noted. The hemoglobin iron content could then be calculated in the following manner:

$$(a) S \times \frac{100}{q} = \mu\text{g per cent hemoglobin in serum,}$$

$$(b) \mu\text{g per cent hemoglobin in serum} \times 0.334 \text{ per cent (Butterfield's factor) } (37) = \mu\text{g per cent hemoglobin iron in serum,}$$

was evidently not great enough to throw the platelets down with the other formed elements. After 15 to 20 minutes of centrifugation, therefore, coagulation of the cell-free plasma occurred. The fibrin of the clot, however, was whirled down onto the surface of the packed red and white cells almost as rapidly as it was formed. There it made a white, rubbery, button-like partition between the serum and the underlying cellular elements, and served to keep the red cells from being disturbed while the serum was removed with a pipette.

<sup>2</sup> The term "serum," rather than "plasma," is used advisedly. The speed at which blood was centrifuged

where  $\mu\text{g}$  = micrograms,  
 $S$  = micrograms of hemoglobin which reagent  
 was just capable of detecting,  
 $q$  = smallest quantity of serum which just gave  
 a perceptible color.

Any colorimetric method which uses as its end-point the smallest amount of any reacting substance which, under given conditions, will just form a perceptible color with a chromophore group is open to obvious objections and to considerable error. The described benzidine method, however, accomplishes the end of demonstrating the fact that only a small amount of the iron present in plasma or serum samples prepared as described is hemoglobin iron. It possesses a special advantage in that it is relatively more accurate in the lower ranges of hemoglobin iron concentration than is either the spectroscopic method of Henriques and Roche (6) or the benzidine method of Bing and Hanzal (9). If, for example, the sensitivity of the benzidine dihydrochloride reagent is 0.3 microgram per cent hemoglobin, and the smallest amount of a particular serum preparation which will just develop a color with it is 0.1 cc., then the hemoglobin iron concentration of that serum is only 1 microgram per cent. For all practical purposes, it matters little whether there is 100 per cent variation from this figure. As the hemoglobin concentration becomes higher, the just detectable color is given by smaller amounts of serum, the denominator in the equation (a) above becomes smaller, and the error produced by any error in the reading becomes correspondingly greater. In Table I are presented representative data: 1, illustrative of the accuracy of the method in recovering known amounts of hemoglobin added to serum, and, 2, comparing figures for hemoglobin iron in intentionally hemolyzed specimens obtained by the benzidine method with those obtained by actually observing the increase in total iron. If the hemoglobin iron concentrations are greater than 40 to 50 micrograms per cent, the method has but little value and spectroscopic methods of quantitation would seem to be more applicable.

The most commonly used method for differentiating between hemoglobin and non-hemoglobin iron in serum eliminates the hemoglobin by precipitating it with trichloroacetic acid. The actual iron determination is then made on the protein-free filtrate of the serum sample being analyzed.

TABLE I

*Accuracy of the benzidine method for determination of hemoglobin iron present in serum or plasma*

I. Recovery of added known quantities of hemoglobin

Hemoglobin iron in serum	Hemoglobin iron added	Total hemoglobin iron		Total amount recovered
		Calculated	Determined	
micrograms per cent	micrograms per cent	micrograms per cent	micrograms per cent	micrograms per cent
0.2	1.2	1.4	2.4	2.2
0.2	2.4	2.6	2.5	2.3
0.2	9.9	10.1	10.0	9.8
2.4	2.3	4.7	7.3	4.9
2.4	22.8	25.2	16.7	14.3
2.4	45.6	48.0	36.5	34.1

II. Comparison of iron and benzidine determination of hemoglobin iron

Patient	Diagnosis	Iron determination			Hemoglobin iron determination		
		Serum	Hemolyzed serum	Hemoglobin iron increase	Serum	Hemolyzed serum	Hemoglobin iron increase
		mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent
A. K.	Polycythemia	0.036	0.054	0.018	0.006	0.029	0.023
J. J. Q.	"Normal"	0.117	0.144	0.027	<0.001	0.036	0.036
W. A.	"Normal"	0.113	0.201	0.088	<0.001	0.092	0.092
W. S.	Treated P. A.	0.069	0.131	0.062	<0.001	0.063	0.063
C. M.	"Normal"	0.137	0.512	0.375	<0.001	0.391	0.391
C. M.	"Normal"	0.095	0.102	0.007	<0.001	0.012	0.012
J. J. Q.	After hemorrhage	0.063	0.088	0.025	<0.003	0.017	0.014
L. R.	Iron deficiency	0.046	0.072	0.026	<0.001	0.048	0.048

As pointed out by Fowweather (8) and Tompsett (11), however, appreciable quantities of true serum iron are carried down with the trichloroacetic acid precipitate, probably by a process of coprecipitation, and values so obtained are correspondingly low. The data presented in Tables II and III substantiate Fowweather's conclusions. Iron determinations were made according to the method described later in this paper. Protein-free filtrates of serum were prepared by adding 3 parts of water to 1 part of serum and then 1 part of a 20 per cent aqueous solution of redistilled trichloroacetic acid, permitting the whole to stand for 15 minutes, and then centrifuging at high speed until the precipitate was well packed. By substituting varying amounts of a standard iron solution (0.1 mgm. per cent iron as ferric chloride) for equal amounts of the water portion, it was possible to establish (Table III) the fact that a definite amount of added iron was also carried down with or trapped by the protein precipitate.



TABLE II

*Recovery of serum iron after precipitation of proteins with trichloroacetic acid*

Subject	Total iron in serum	Hemo-globin iron in serum	Non-hemo-globin serum iron	Serum iron recovered in trichloroacetic acid filtrates	
				Amount	Recovery
	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	per cent
E. S. ♂ Polycythemia vera—after bleeding.....	0.039	<0.001	0.039	0.026	66.7
M. B. ♂ Hypochromic anemia.....	0.029	<0.001	0.029	0.024	82.8
W. S. ♂ Pernicious anemia (liver therapy for 2 weeks)	0.057	<0.001	0.057	0.047	82.5
E. H. ♂ "Normal".....	0.065	<0.001	0.065	0.052	80.0
C. V. M. ♂ "Normal".....	0.126	<0.001	0.126	0.101	80.1
A. R. ♀ "Normal".....	0.135	<0.001	0.135	0.127	94.8
A. H. ♂ "Normal".....	0.126	<0.001	0.126	0.108	85.7
O. E. ♀ Aplastic anemia...	0.163	0.002	0.161	0.124	77.0
E. R. ♀ 1 hour after 6.0 grams ferrum reductum—orally.....	0.185	<0.001	0.185	0.121	65.4
C. C. ♂ Aplastic anemia...	0.300	0.004	0.296	0.211	71.3
G. H. ♀ Pernicious anemia of pregnancy.....	0.274	0.004	0.270	0.191	69.9

TABLE III

*Effect of trichloroacetic acid precipitation on recovery of known amounts of iron added to serum*

Subject	Non-hemo-globin serum iron	Serum iron recovered from trichloroacetic acid filtrate		Iron added	Total iron determined in filtrate		Added iron recovered	
		Amount	Recovery		Amount	Recovery	Amount	Recovery
		mgm. per cent	per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	per cent
E. S. ♂ Polycythemia vera	0.039	0.026	67	0.033	0.058	0.032	97	
	0.039	0.026	67	0.067	0.074	0.048	72	
	0.039	0.026	67	0.133	0.122	0.096	72	
C. V. M. ♂ "Normal"	0.095	0.083	87	0.100	0.171	0.088	88	
	0.124	0.115	93	0.050	0.157	0.042	84	
L. K. ♀ "Normal"	0.124	0.115	93	0.200	0.275	0.161	81	
J. J. Q. ♂ "Normal"	0.117	0.111	95	0.050	0.155	0.044	88	
C. V. M. ♂ "Normal"	0.110	0.095	86	0.100	0.168	0.073	73	
F. F. ♂ Pernicious anemia in relapse	0.221	0.152	69	0.050	0.186	0.034	68	
	0.221	0.152	69	0.100	0.219	0.067	67	

A critical analysis of these two tables demonstrates that there is no true constancy about the amount of serum iron lost in the precipitation process, and that this quantity may vary from only 5 or 10 per cent to as much as 40 per cent. It is interesting to note that the loss recorded in the values for the two cases of aplastic anemia is comparatively high. The factors which influence the amount of iron lost along with the protein precipitate are, however, not at present apparent.

TABLE IV

*Recovery of iron added to serum*

Subject	Amount of serum digested	Total iron in serum	Iron added as ferric chloride*	Total iron determined	Recovery
	cc.	mgm. per cent	mgm. per cent	mgm. per cent	per cent
Pernicious anemia ♀ (early remission)...	10	0.057	0.050	0.107	100
Hypochromic anemia ♂.....	10	0.028	0.100	0.128	100
Pernicious anemia ♂ (early remission)...	10	0.040	0.200	0.237	99
Normal ♂.....	10	0.117	0.050	0.167	100
Normal ♂.....	10	0.095	0.100	0.197	102
Normal ♂.....	10	0.095	0.200	0.288	97
Normal ♂.....	10	0.158	0.050	0.206	97
Aplastic anemia ♂...	5	0.200	0.100	0.299	99
Aplastic anemia ♂...	5	0.200	0.200	0.403	102

\* Iron added as a solution of ferric chloride (prepared from standard iron wire) which contained 0.100 mgm. per cent iron. Thus, to add 0.050 mgm. per cent iron to 10 cc. serum, 5 cc. of the iron solution was added; or to add 0.100 mgm. per cent iron to 10 cc. serum, 10 cc. of the iron solution was added; or to add 0.200 mgm. per cent iron to 10 cc. serum, 20 cc. of the iron solution was added.

TABLE V

*Serum iron determinations on "normal" adults*

Males					Females				
Subject number	Hematocrit	Serum iron			Subject number	Hematocrit	Serum iron		
		Total iron in serum	Hemo-globin iron in serum	Non-hemo-globin serum iron			Total iron in serum	Hemo-globin iron in serum	Non-hemo-globin serum iron
	per cent	mgm. per cent	mgm. per cent	mgm. per cent		per cent	mgm. per cent	mgm. per cent	mgm. per cent
1	46	0.112	0.008	0.104	1	45	0.119	<0.001	0.119
2	44	0.098	<0.001	0.098	2	42	0.110	0.028	0.072
3	44	0.162	0.003	0.159	3	45	0.087	<0.001	0.087
4	45	0.112	0.002	0.110	4	45	0.120	<0.001	0.120
5	46	0.126	0.032	0.094	5	41	0.093	<0.001	0.093
6	49	0.115	<0.001	0.115	6	44	0.114	0.016	0.098
7	46	0.157	0.024	0.133	7	39	0.124	<0.001	0.124
8	46	0.126	<0.001	0.126	8	42	0.108	<0.001	0.108
9	45	0.174	<0.001	0.174	9	42	0.145	0.003	0.142
10	45	0.118	<0.001	0.118	10	43	0.065	<0.001	0.065
11	48	0.096	<0.001	0.096	11	44	0.058	<0.001	0.058
12	47	0.122	<0.001	0.122	12	42	0.083	0.003	0.080
13	45	0.164	<0.001	0.164	13	42	0.085	<0.001	0.085
14	45	0.115	<0.001	0.115	14	43	0.114	<0.001	0.114
15	46	0.121	0.026	0.095	15	43	0.099	<0.001	0.099
Maximum		0.174			Maximum		0.142		
Minimum		0.094			Minimum		0.058		
Average		0.1215			Average		0.0976		

Barkan (1, g) showed that the iron in serum or plasma is not ultrafiltrable, but that it is made so when acidified with weak hydrochloric acid. He incubated the acidified plasma for 24 hours at 37.5° C. and then submitted it to a process of

TABLE VI  
*Iron content of human serum or plasma*

Investigators	Material used	Subjects	Number	Results <i>mgm. per cent</i>
1. Starkenstein and Weden (3)....	Protein free serum (trichloracetic acid)	Adults	5	0.690*(0.570-0.790)
2. Riecker (21a).....	Protein free serum (trichloracetic acid)	Adults	40	1.110(0.9-1.4)
3. Marlow and Taylor (7).....	Protein free serum (defibrinated; trichloracetic acid)	Adults	5	0.400-0.700
4. Warburg and Krebs (19).....	Whole serum. Catalytic method	Adults	4	0.084(0.067-0.116)
5. Langer (22).....	Protein free serum (trichloracetic acid)	Adults	20	0.110(0.050-0.180)
6. Guthmann, Brückner, Ehrenstein and Wagner (23).....	Whole serum (ultrafiltration)	Men	22	0.065(0.042-0.098)
		Women	26	0.068(0.035-0.098)
7. Locke, Main and Rosbash (24)...	Protein free serum (trichloracetic acid)	Men	8	0.100 $\pm$ 0.015
		Women	9	0.077 $\pm$ 0.015
8. Barkan (1g).....	Whole plasma and serum	Adults	15	0.105*(0.056-0.140)
9. Fowweather (8).....	Hemoglobin free whole plasma	Men	10	0.125(0.095-0.180)
		Women	10	0.105(0.060-0.156)
10. Tompsett (11).....	Thiolactic acid-protein free serum	Adults	10	0.120-0.220
11. Thoenes and Aschaffenburg (25)	Protein free serum (trichloracetic acid)	Infants at birth	12	0.173(0.134-0.291)
		Children 13 days to 9 years	60	0.040-0.196
12. Roosen-Runge (26).....	Whole serum-ultrafiltration	Adults	10	0.110
13. This study.....	Whole serum (hemoglobin present quantitated)	Men	15	0.121(0.094-0.174)
		Women	15	0.097(0.058-0.142)

\* Not "normal" subjects.

ultrafiltration. Under these conditions, the iron of the hemoglobin molecule does not become ionizable; and the iron which passes through the filter represents the non-hemoglobin plasma iron. That the values obtained by Barkan with such a method are in close agreement with those obtained by other investigators is apparent from Table VI. We have been able to confirm Barkan's observations by acidifying serum with 0.2 N HCl and dialyzing it at body temperature in a cellophane membrane against 0.1 N HCl for 48 hours. Values so obtained have agreed remarkably with those arrived at by actual digestion of the whole serum or plasma.

It is, therefore, possible to: 1, prepare serum or plasma which is, for all practical purposes, free of hemoglobin; 2, quantitate small amounts of contaminating hemoglobin iron in serum; 3, precipitate any contained hemoglobin along with serum proteins with trichloracetic acid; and 4, separate the non-hemoglobin serum iron from any hemoglobin present by ultrafiltration or dialysis after acidification with 0.1 N HCl. The method of precipitation is open to objection in that a portion of the non-hemoglobin iron is carried down with the protein precipitate, and the re-

sultant serum iron values are correspondingly low. With these various methods of eliminating or quantitating hemoglobin iron in serum, the non-hemoglobinous character of the quantities determined by the technique immediately to be described seems to be established.

## II. The determination of plasma and serum iron

Since only 50 to 180 micrograms of non-hemoglobin iron are normally present in 100 cc. of human serum, and since it is frequently not practical to obtain more than 10 to 15 cc. of serum for analysis, methods used to quantitate serum iron must be capable of measuring 6 to 10 micrograms or less of the metal. Titrimetric methods of sufficient sensitivity to accomplish this result have so far not been developed, and chief reliance has been placed on colorimetric determinations. Most satisfactory of these, particularly because of the greater intensity of the color developed, is that which depends on the red color of ferric thiocyanate.

The thiocyanate method used in these investigations is a modification of Kennedy's (12) procedure.

1. All glassware used was washed with concentrated HCl and rinsed with water redistilled from an all glass West distillation apparatus (13). This double glass-distilled water was used throughout.

2. Reagents used: (a) reagent grade sulfuric acid (Grasselli); (b) 30 per cent hydrogen dioxide (Baker); (c) reagent grade nitric acid (Grasselli); (d) iso-amyl alcohol (Merck Blue label); and (e) potassium thiocyanate (Merck Blue label). The trichloroacetic acid used for the determinations listed in Tables II and III was distilled under reduced pressure from a modified West distillation unit.

3. Digestion. Either 10 or 15 cc. of measured serum were pipetted into a 300 cc. Kjeldahl flask along with 5 cc. of concentrated sulfuric acid and digestion carried out over a moderate Bunsen flame. After charring and the usual rapid evolution of  $\text{CO}_2$  had occurred, the digestion mixture was permitted to cool, 4 to 5 cc. of 30 per cent hydrogen peroxide were added and the mixture again heated. Repeated additions of hydrogen peroxide—9 to 12 cc. usually sufficed—were made until the solution became colorless. The flask was then rinsed down with the double glass-distilled water and reheated to insure complete breakdown of the peroxide. Several drops of concentrated nitric acid were added while the sulfuric acid digest mixture was still hot (70 to 100° C.), and the whole transferred to a 50 cc. glass stoppered volumetric flask.

4. Colorimetric comparison. Twenty cc. aliquot portions of the sample were pipetted into 60 cc. cylindrical glass-stoppered separatory funnels and overlaid with 10 cc. iso-amyl alcohol. Five cc. of a 20 per cent KSCN solution were then blown into the funnels and the mixture immediately shaken. The colored amyl alcohol layer was separated and colorimetrically compared with a similarly prepared iron standard amyl alcohol solution of ferric thiocyanate. Occasionally, clouding of the amyl alcohol occurred, but a second shaking before separation from the aqueous layer always sufficed to obviate this difficulty.

The iron standard was prepared by placing exactly 0.5 gram of reagent iron wire in a one liter volumetric flask calibrated by the United States Bureau of Standards, dissolving it in concentrated HCl and  $\text{HNO}_3$  (at room temperature for 2 to 3 days) and then diluting it to volume. Two cc. of this solution diluted to one liter contained 0.1 mgm. per cent ferric iron, and varying quantities of it were digested in the manner described above (using comparable amounts of reagents), made up to a 50 cc. volume, and 20 cc. aliquot portions taken for the preparation of the actual colorimetric standards. Standard solutions of iron which varied in iron content from 0.005 mgm. of iron per 50 cc. to 0.05 mgm. of iron were kept on hand and that one selected for any particular determination which most closely approximated the iron concentration of the "unknown."

The total amount of iron present in the quantities of reagents used for one analysis varied between 10 and 12 micrograms per cent. Since comparable amounts of re-

agents were added to the standards, the error thus introduced was practically negligible except for serum iron concentrations of less than 0.030 mgm. per cent.

#### 5. Calculation:

$$\frac{S}{U} \times \frac{100}{\text{cc. of serum}} \times \text{Fe} = \text{mgm. per cent iron,}$$

where  $S$  = reading of the standard;

$U$  = reading of the unknown;

Fe = quantity of iron present in 50 cc. of the iron standard prepared as described above.

6. Precipitation with trichloroacetic acid. For the determination of iron in a trichloroacetic acid protein-free filtrate of serum or plasma, 1 part of serum was diluted with 3 parts of water. To this was added 1 part 20 per cent trichloroacetic acid with gentle shaking. The mixture was permitted to stand for 15 minutes and the protein precipitate centrifuged down. An aliquot portion of the clear supernatant, protein-free solution was then digested with  $\text{H}_2\text{SO}_4$  and the iron content determined as described under 3, 4 and 5 above.

That the iron loss noted in such "filtrates" was due to a process of co-precipitation and not to partial volatilization of the iron as ferric chloride, was apparent from the following experiment. On repeated occasions, 10 cc. of serum were placed in a 300 cc. Kjeldahl digestion flask along with 30 cc. of water and 10 cc. of 20 per cent trichloroacetic acid, and the whole digested. The amount of iron recovered was always found to be identical with that recovered from the simple digestion of 10 cc. of the same serum sample.

The addition of nitric acid to the hot digestion mixture insures the complete oxidation of iron to the ferric state (14). Amyl alcohol was used to extract the iron thiocyanate color in preference to the more volatile ether, acetone, and ethylacetate (16). The red color of iron thiocyanate was found to be stable in amyl alcohol solutions for at least fifteen minutes, so that there was ample time in which to make the colorimetric comparisons. Attention has frequently been called to the necessity for regulating the acidity of the solutions in which the ferric thiocyanate color is developed and from which it is extracted by amyl alcohol or any one of the other extractives just mentioned (14, 15, 17). However, under the conditions described, it was found that the color developed was constant in an acidity range of from 1 to 5 N  $\text{H}_2\text{SO}_4$ . Furthermore, the quantities of serum or plasma digested were relatively constant and the amount of  $\text{H}_2\text{SO}_4$  used, standardized. The normality of the digestion mixtures after having been diluted to 50 cc. has never been found to be outside the limits of 2 to 4 N.

The accuracy of the method is in part defined in Table IV in which the recovery of known amounts of iron added to serum prior to digestion is recorded. It is apparent that a recovery of 96 to 102 per cent was achieved, an accuracy entirely adequate for the study of plasma and serum iron values in health and in disease.

### III. Normal values for serum or plasma iron

Determinations of serum iron in various laboratory and domestic animals have been made by Fontès and Thivolle (18), Starkenstein and Weden (3), Henriques and Roche (6), Warburg and Krebs (19), Barkan (1, d), Abderhalden and Möller (20), and others. The observations recorded are not sufficiently numerous to merit tabulation for comparison. In general, the values reported for animals are 50 to 100 per cent higher than the normal human mean.

Part of the reluctance to accept as valid the recent work on non-hemoglobin serum and plasma iron has grown out of the fact that normal levels reported by different investigators, particularly those working with human subjects, have shown as much as 1000 per cent variation. In Table VI, however, in which is presented in summary form the various values for normal human plasma iron so far reported, it is apparent that the results of only three groups of investigators (3, 7, 21) are out of line with the rest. If these three reports are omitted from consideration, one is impressed with the unanimity of the data presented by the remaining workers, an agreement that is all the more remarkable in that at least four different techniques were employed.<sup>3</sup> The reasons for the higher figures given in the three dissenting reports listed above are not obvious from a review of the available data. However, the great similarity in the normal zonal ranges obtained by nine groups of investigators constitutes sufficient diverse confirmation, we feel, to permit the following two conclusions: 1, in normal human subjects, the serum or plasma iron level varies from approximately 0.050 to 0.180 mgm. per cent; and, 2, the mean value for serum iron in normal males

is approximately 0.020 to 0.030 mgm. per cent higher than the mean value for normal females. Table V tabulates the determinations on each of the thirty normal subjects studied by us and summarized in Table VI.

### IV. Comparison between plasma and serum iron values

It is of theoretical interest, as Fowweather has indicated, to compare serum and plasma iron values since there is the possibility that some of the plasma iron may be removed in the clot when serum is formed. We have found that when blood obtained by venipuncture is permitted to run from an 18 gauge needle directly into a parafined centrifuge tube and immediately centrifuged at 2500 r.p.m., the erythrocytes and leukocytes are packed before coagulation takes place. The plasma, in fact, usually does not coagulate for 15 to 20 minutes; and as the fibrin forms, it is thrown down as a thin layer on top of the formed elements. For the present study, two tubes of blood were collected and centrifugation immediately begun. After 12 minutes, one of the tubes was taken from the centrifuge, the unclotted plasma removed, and 10 cc. of it pipetted promptly into a Kjeldahl digestion flask. Coagulation occurred within a few minutes after the transfer, but all of the constituents of the plasma were obviously digested. The second tube was removed from the centrifuge at the end of an hour. Complete coagulation had occurred within this period, and the fibrin had been centrifuged down. A 10 cc. quantity of the serum was then removed for digestion. The iron values obtained for these serum and plasma specimens collected simultaneously agree with each other well within the limits of error of the method (Table VII), and demonstrate the fact that no appreciable quantity of iron is removed from plasma when coagulation occurs.

Abderhalden and Möller (20) have raised the question as to whether any iron is added to serum from the cells when whole blood is permitted to coagulate. In order to study this possibility, two tubes of blood were collected from subjects as above described. One of these was immediately centrifuged for 60 minutes and the serum collected in the manner that was routine throughout these studies. The second tube was per-

<sup>3</sup> 1. The catalytic method of Warburg.

2. The ultrafiltration of serum before digestion.

3. Thiolaetic acid method.

4. Various modifications of the thiocyanate method.

TABLE VII  
Comparison between serum and plasma iron values

Subject	Whole serum			Whole plasma			Variation
	Total iron present	Hemo-globin iron	Non-hemo-globin iron	Total iron present	Hemo-globin iron	Non-hemo-globin iron	
	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	per cent
E. S. ♂ Polycythemia vera after bleeding....	0.020	0.002	0.027	0.028	0.0015	0.027	0
L. R. ♀ Idiopathic hypochromic anemia.....	0.051	<0.001	0.054	0.052	<0.001	0.052	-3.8
F. F. ♂ Pernicious anemia (remission).....	0.110	0.003	0.116	0.118	<0.001	0.118	+1.7
C. V. M. ♂ "Normal"....	0.148	0.004	0.144	0.140	<0.001	0.140	-2.8
A. K. ♂ 6 hours after 10 grms ferrum reductum by mouth.....	0.218	<0.001	0.218	0.223	0.001	0.222	+1.8
C. C. ♂ Aplastic anemia.	0.313	0.002	0.311	0.308	<0.001	0.308	-0.9

mitted to stand at room temperature for 40 minutes, by which time complete coagulation had occurred. The clot was then gently loosened and the serum obtained by centrifugation. So close an agreement was obtained between the iron content of the serum specimens prepared in these two ways (Table VIII) that it has been possible

TABLE VIII  
Comparison of the non-hemoglobin iron content of serum prepared by: I. Immediate centrifugation and separation from the formed elements; and II. Centrifugation after complete coagulation of the whole blood.

Subject	Serum obtained by immediate centrifugation			Serum obtained after complete coagulation of the whole blood (40 minutes after venesection)			Variation
	Total iron present	Hemo-globin iron	Non-hemo-globin iron	Total iron present	Hemo-globin iron	Non-hemo-globin iron	
	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	per cent
A. K. ♂ Polycythemia vera after bleeding....	0.022	<0.001	0.022	0.022	<0.001	0.022	0
W. S. ♂ Pernicious anemia (after liver).....	0.057	<0.001	0.057	0.057	<0.001	0.057	0
L. R. ♂ Pernicious anemia (after liver).....	0.060	<0.001	0.060	0.057	<0.001	0.057	-5.0
C. V. M. ♂ "Normal"....	0.098	<0.001	0.098	0.096	<0.001	0.096	-2.0
J. J. Q. ♂ "Normal"....	0.117	<0.001	0.117	0.123	0.004	0.119	+1.7
J. R. ♂ "Normal".....	0.158	<0.001	0.158	0.150	<0.001	0.150	-5.3
O. E. ♂ Aplastic anemia..	0.163	0.001	0.162	0.166	0.001	0.165	+2.0

to conclude that non-hemoglobin iron is not increased in serum in appreciable amounts when whole blood coagulates.

### V. Summary

Evidence which tends to establish plasma or serum iron as of non-hemoglobinous origin has been presented. Reference to four different

chemical methods by which the iron in sera may accurately be determined has been made, and a detailed description of the colorimetric thiocyanate technique used in these studies has been given. The normal zonal range for human plasma iron was found to be approximately 0.050 to 0.180 mgm. per cent. The average value for normal men proved to be slightly higher than the average for normal women. No essential difference in the plasma and serum iron values was detected.

The chemical relationships and nature of serum iron are not well understood. It is known that serum iron does not pass through a semi-permeable membrane unless altered as by acidification (1, g); the iron, therefore, is not in an ionized state. It may be present as a component of a complex radical. Starkenstein and Harvalik (46) believe it to be in combination with the protein of the serum, and, more specifically, with globulin. Little doubt remains that the iron in serum is in the trivalent state (46).

### B. "EASILY SPLIT-OFF" BLOOD IRON

Barkan (1, a, b) and Lintzel (2, a), working independently, each made the observation that when whole blood is subjected to the action of various of the digestive ferments, approximately 5 to 10 per cent of the blood iron is changed to an ionized form. They later discovered that the same "splitting-off" action is exerted either by dilute acids or by dilute bases alone. Lintzel and Radeff (2, b) were able to "split-off" this ionizable iron from preparations of crystalline hemoglobin and came to believe that the metal was derived directly from the dissociation of the hemoglobin molecule. Barkan repeated Lintzel's observations, but on one occasion (1, b) obtained a preparation of crystalline hemoglobin, preserved constantly under alcohol, from which only traces of iron could be dissociated or "split-off" by dilute hydrochloric acid. This and other data led him to believe that "das leicht abspaltbare Bluteisen" fraction constituted a third type of blood iron, distinct from either hemoglobin or plasma iron. In subsequent well controlled, well directed researches, Barkan (1, c) has attempted further to establish the identity of "easily split-off" iron and has tentatively ascribed to it the

function of iron transportation in the blood stream (1, *l*).

That some 5 to 10 per cent of the iron in blood is ionized by dilute acids, there can be no doubt. It is not as yet certain, however, that the "easily split-off" fraction is a non-hemoglobinous form of blood iron even though it must be admitted that the experimental evidence accumulated by Barkan points definitely to that conclusion.

It is unfortunately true that "the precise ascertainment of the Fe content of purified human hemoglobin is a problem that awaits solution" (Peters and Van Slyke (27)). Nevertheless, a close approximation to that content has probably been obtained by Hüfner (28) and by Morrison and Hisey (29) who found that hemoglobin contained 0.34 per cent iron. With the use of this figure, it is possible to calculate from the whole blood iron that amount of hemoglobin which would be present per unit of peripheral blood if all of the iron were present in hemoglobinous combination.<sup>4</sup> The calculated hemoglobin value, then, might be compared with that obtained by actual determination with either oxygen capacity or acid hematin methods. If there should be agreement between the determined and the calculated hemoglobin values, it would be fair to assume that all of the blood iron (except that present in the serum) is hemoglobin iron. Such an agreement would constitute presumptive evidence that the "easily split-off" iron is dissociated from the hemoglobin molecule *per se*. If, on the other hand, it should be found that more iron is present in the blood stream than can be accounted for by the hemoglobin present, then it would seem that Barkan's point of view would be substantiated, and "easily split-off" iron would have to be considered a form of non-hemoglobin blood iron. As a matter of fact, many such comparisons have been made. Most observers (12, 30 to 36) have obtained agreements of 1 per cent or less between calculated and determined hemoglobin values. But Klumpp (38), using the oxygen capacity gasometric method for the determination of hemoglobin, and Josephs (39), using an acid hematin method, found more iron present in the

blood stream than would have been the case had it all been present as a component of the hemoglobin molecule. Until more definite agreement is attained, therefore, no conclusions may be drawn from this type of reasoning.

"Easily split-off" blood iron has been determined in several ways. Barkan (40) acidified whole blood with 0.8 per cent hydrochloric acid, incubated it for 24 hours at 37.5° C., and then passed it through an ultrafilter, using a collodion membrane. He showed that the ionization or "splitting-off" process was not immediate, but that it was practically complete at the end of 24 hours. Dominici (4) and Starkenstein and Weden (3) heated hydrochloric acidified whole blood to boiling and then precipitated the proteins with trichloroacetic acid. Winegarden and Borsook (41) obtained the fraction by placing 5 cc. of blood and 5 cc. of 0.2 N HCl in cellophane bags and dialyzing against 0.1 N HCl at 37.5° C. for 24 hours, changing the outside acid once at the end of 6 to 8 hours. Olesk (43) and Schwarz and Deckert (42), using the ultrafiltration method, obtained values which are in close agreement with those of Barkan. Dominici (4) made the interesting observation that 0.1 N H<sub>2</sub>SO<sub>4</sub> will "split-off" considerably more iron than 0.1 N HCl.

The method employed in this study is similar to the one used by Winegarden and Borsook. Cellophane tubing was cut in 12 inch lengths and washed for 24 hours in iron-free 0.2 N HCl. Each end of the cellophane strip was then slipped over a short section of glass tube (13 mm. diameter, 2 inches long), made fast with a rubber band, and the whole washed with iron-free water. Into one of the open ends, 5 cc. of whole oxalated blood and 5 cc. of 0.2 N HCl were pipetted. The membrane was then suspended in a 250 cc. beaker containing 100 cc. 0.1 N HCl. The dialysis was carried out at 37.5° C. At 24 hour intervals for 4 days, the outside HCl was removed and replaced by a fresh 100 cc. At the end of the dialyzing period, the total amount of HCl which had been used outside the membrane was made up to a volume of 500 cc. Aliquot portions of this were digested in Kjeldahl flasks and the iron determinations completed in a manner similar to that described for the determination of plasma or serum iron. Under these conditions, only about

<sup>4</sup> The serum iron is relatively so small that it may, for the sake of convenience, be omitted from the present consideration.



65 to 75 per cent of the ionized iron was obtained after a 24 hour period of dialysis; over 95 per cent was dialyzed in 4 days. Detectable, but very small amounts of iron could be obtained in the HCl surrounding the membrane for 2 or 3 days more. The method was standardized, however, for a 4 day dialyzing period.

The average value obtained by Barkan on twenty-two human subjects in Frankfort was 1.42 mgm. per cent. Schwarz and Deckert (42), working in Hamburg, found a variation of from 1.26 mgm. per cent to 1.68 mgm. per cent with an average of 1.47 mgm. per cent. Olesk (43) working in Barkan's laboratory in Esthonia, observed an average value of 1.85 mgm. per cent on 73 subjects, with range extremes of 1.39 to 2.23 mgm. per cent. He reported that the average figure for males was slightly higher than the average for females. Dominici (4), however, using sulfuric instead of hydrochloric acid, noted a variation from 0.92 to 3.12 mgm. per cent on twelve subjects, and obtained an average of 2.22 mgm. per cent. This last observation by Dominici to the effect that greater amounts of iron are "split-off" by sulfuric than by hydrochloric acid led us to make determinations of the "easily split-off" iron fraction on the same sample of blood using different acids in several concentrations. In Table IX are recorded several such experiments in which hydrochloric, sulfuric, and nitric acids were

TABLE IX

"Easily split-off" blood iron as determined with varying concentrations of different mineral acids

Sub- ject	HCl				H <sub>2</sub> SO <sub>4</sub>		HNO <sub>3</sub>	
	0.05 N	0.1 N	0.2 N	0.35 N	0.1 N	0.2 N	0.1 N	0.2 N
	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent
C. V. M....		2.190	1.841	1.623	3.614	3.614	3.846	3.670
W. R. A....		1.623	1.238	1.000	3.061	3.061	3.158	
J. J. Q....	3.300	2.714	1.515	1.316	3.822	4.028	3.750	3.530
P. B.....	1.851	1.712	1.562		2.608	2.631		
H. W.....	2.740	2.362	2.069		3.658	3.691		

used. It is immediately apparent that: 1, sulfuric and nitric acids "split-off" approximately the same amount of iron, an amount that is considerably greater than that "split-off" by hydrochloric acid; 2, sulfuric and nitric acids dissociate or ionize approximately the same amounts of iron whether used in concentrations of 0.1 or 0.2 N; and 3, increasing the concentration of hydrochloric acid tends to decrease, rather than to increase, the amount of iron it dissociates.

"Easily split-off" iron values on twenty "normal" human subjects were determined with both 0.1 N HCl and 0.1 N H<sub>2</sub>SO<sub>4</sub> according to the method of dialysis described above. The results, together with the pertinent hematological data, are tabulated in Table X. The values are somewhat higher than those reported by the various European workers. With 0.1 N HCl, for instance, an

TABLE X

"Easily split-off" iron content of "normal" human blood

Males						Females					
Sub- jects	Hematological data			"Easily split-off" iron		Sub- jects	Hematological data			"Easily split-off" iron	
	R.B.C.	Hb.	Cell vol- ume	0.1 N HCl	0.1 N H <sub>2</sub> SO <sub>4</sub>		R.B.C.	Hb.	Cell vol- ume	0.1 N HCl	0.1 N H <sub>2</sub> SO <sub>4</sub>
	millions	grams	per cent	mgm. per cent	mgm. per cent		millions	grams	per cent	mgm. per cent	mgm. per cent
S. R.....	5.22	15.6	44.0	2.186	4.397	R. B.....	4.61	12.5	44.0	3.154	4.786
B. H.....	4.54	16.5	47.4	2.167	4.340	M. H.....	4.47	14.1	40.0	2.520	4.318
W. H.....	4.90	16.3	40.0	2.286	3.707	M. M.....	4.32	13.2	38.0	1.963	4.436
W. D. P....	5.16	15.0	44.0	2.613	4.111	E. M.....	4.69	13.5	41.5	2.934	4.634
J. J. Q.....	5.85	16.4	46.5	2.600	3.708	E. M. W.	5.13	9.7	32.5	2.497	4.334
J. R.....	4.92	15.2	44.5	2.190	4.026	L. R.....	4.42	13.3	40.0	2.841	4.859
D. W.....	4.42	14.8	40.2	2.884	4.142	G. H.....	4.66	11.7	37.0	2.108	3.576
C. V. M.....	5.21	15.6	45.2	2.076	3.500	M. H.....	3.80	12.0	37.8	2.301	3.849
W. R. A.....	4.86	14.3	42.0	1.499	2.947	M. A.....	4.56	12.8	38.0	2.676	4.671
H. W.....	3.89	13.9	41.0	2.362	3.658	M. W.....	4.02	12.2	36.5	2.305	4.366
		Minimum		1.499	2.947			Minimum		1.963	3.576
		Maximum		2.884	4.397			Maximum		3.154	4.786
		Average		2.286	3.854			Average		2.530	4.283

average figure of 2.408 mgm. per cent was obtained, and the range extended from 1.499 to 3.154 mgm. per cent. The only investigator who has used sulfuric acid is Dominici, and his technique is not strictly comparable to the one used here. The "easily split-off" iron determinations made with 0.1 N sulfuric acid averaged 4.068 mgm. per cent and varied from 2.947 to 4.786 mgm. per cent.

Barkan and Berger (1, *e*) also observed that approximately 60 to 70 per cent of the "easily split-off" iron fraction is bound by CO in such a manner that it is protected against the "splitting-off" action of acids. The whole of this sub-fraction "E" is contained in the erythrocytes and is reported to have greater affinity for CO than does hemoglobin, i.e., with partial saturation of the blood, the gas unites with a greater per cent of "easily split-off" iron than with hemoglobin. Schwarz and Deckert (42) and Olesk (43) have noted that the CO absorbed by heavy smokers is sufficient to bind a portion of the "easily split-off" iron, and caution that such determinations should be made after a period of "tobacco-fasting." The 30 to 40 per cent "easily split-off" iron not bound by CO has been termed (1, *e*) the E' sub-fraction and has been observed to be present in both the red cells and in plasma (all of the iron in plasma is determined as a part of this E' portion).

Tompsett (11) with a thioglycolic acid method and McIntosh (44) by precipitating whole blood with trichloroacetic acid attempted to determine the inorganic iron of whole blood and obtained values of about 1.0 mgm. per cent. It is difficult, of course, to know how much of the so-called inorganic iron fraction which McIntosh measured was carried down with the protein precipitate as has been shown to occur with plasma iron (Tables II and III). Barkan (1, *i*) has presented evidence to show that the "easily split-off" blood iron is not an inorganic form of iron.

#### SUMMARY

A satisfactory understanding of the chemical nature of "easily split-off" blood iron has not as yet been attained. Certain it is that approximately 5 to 10 per cent of whole blood iron is "split-off" or dissociated from its usual combination by the action of dilute acids. With the

method of dialysis used in these investigations, we have found the average value of this fraction on twenty "normal" subjects to be approximately 2.3 to 2.5 mgm. per cent when acidification was accomplished with 0.1 N HCl. The figures extended from 1.499 mgm. per cent to 3.154 mgm. per cent. When sulfuric (or nitric) acid was substituted for hydrochloric, however, the values obtained were somewhat higher and ranged from 2.947 to 4.786 mgm. per cent. The significance of the greater "splitting-off" ability of sulfuric and nitric acids is not as yet entirely clear. There are no strictly comparable values in the literature with which those of the iron "easily split-off" by sulfuric acid may be compared. Those workers who have used hydrochloric acid and Barkan's method of ultrafiltration have obtained range extremes of from 1.2 to 2.23 mgm. per cent on "normal" human subjects.

"Easily split-off" iron may be a form of non-hemoglobin blood iron, as Barkan believes, but further proof is necessary before we may accept this fraction as composed of iron unassociated with the hemoglobin molecule. Since evidence has accumulated which tends to show that there is more than one kind of hemoglobin in human blood (45)—and, possibly, in the blood of animals as well—, the question naturally arises as to what relationship, if any, "easily split-off" iron may have to the hemoglobin form or forms which may be present in relatively small concentration. Barkan and Schales (1, *n*) have pointed out that since "easily split-off" iron and hemoglobin react similarly to adsorption on argillaceous earths and to cataphoresis, and since both combine readily with carbon monoxide, the two molecules are probably closely related structurally. Dilute acids do ionize or "split-off" a certain proportion of the whole blood iron, but identification of the molecule from which this dissociation takes place is yet to be accomplished.

#### CONCLUSIONS

##### I. Plasma or serum iron

- A. Plasma or serum iron is a non-hemoglobinous form of blood iron.
- B. Various methods for the determination of plasma iron have been described, the most satisfactory of these being the colorimetric method which



determines the iron present as ferric thiocyanate in an amyl alcohol solution.

C. The normal zonal range for human plasma iron is approximately 0.050 to 0.180 mgm. per cent. The average value for men is slightly higher than the average for normal women.

D. Plasma and serum iron values are essentially identical.

## II. "Easily split-off" blood iron

A. When whole blood is acidified, 5 to 10 per cent of the total iron present becomes ionized so that it will pass through a semipermeable membrane.

B. With Barkan's method of ultrafiltration through a collodion membrane after acidification of the blood with 0.8 per cent HCl and incubation at body temperature for 24 hours, the amount of "easily split-off" iron in normal human subjects has been found to vary from 1.2 to 2.25 mgm. per cent. With hydrochloric acidification and the method of dialysis (through a cellophane membrane) used in these studies, a range for "easily split-off" blood iron of from 1.499 to 3.154 mgm. per cent was obtained on twenty "normal" human subjects.

C. Sulfuric and nitric acids "split-off" considerably more iron than does hydrochloric acid (range 2.947 to 4.786 mgm. per cent). Values obtained with sulfuric and nitric acidification are in close agreement.

D. With increases in concentration of hydrochloric acid, the amount of iron "split-off" progressively decreases. Changes in concentration from 0.1 to 0.2 N sulfuric or nitric acids did not alter the values obtained.

E. The work of Barkan points to the conclusion that "easily split-off" iron is an organic, non-hemoglobinous form of iron, but further proof is needed before we may accept the

fraction as being unassociated with the hemoglobin molecule.

III. The further validity of these conclusions has been suggested by the application of the above principles in selected clinical cases which are being presented coincidentally.

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# STUDIES IN IRON TRANSPORTATION AND METABOLISM. II. THE MECHANISM OF IRON TRANSPORTATION: ITS SIGNIFICANCE IN IRON UTILIZATION IN ANEMIC STATES OF VARIED ETIOLOGY<sup>1</sup>

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A growing interest in the mechanism by which iron is transported in the blood stream has been stimulated by the desire to understand more fully this essential phase of iron metabolism in the mammalian body. With the clarification of this important detail a more complete understanding of normal erythropoiesis and of the iron deficiency states may be anticipated.

Despite a wide variety of observations reported in the literature, an entirely satisfactory and acceptable experimental definition of the manner in which iron transportation is effected has not as yet been presented. A. B. Macallum (1), Ehrlich and Lazarus (2), and Proescher and Arkush (3) observed that histologically many erythrocytes give an iron reaction. They assumed, therefore, that free iron, not identified with the hemoglobin molecule, is present in at least a portion of the red cells. Macallum postulated that iron is transported by the erythrocytes, and Proescher and Arkush further suggested that this may be accomplished through a loose combination with the lecithin of the erythrocyte corpuscular membrane. Other investigators (4 to 14) have studied the iron content of plasma and serum in the hope that some light might be shed on this problem of mineral transport, but their researches have not yielded conclusive information. Barkan (15, *a* and *b*) has assigned to the "easily split-off" blood iron fraction the function of iron transportation and considers plasma iron as the medium of exchange between the tissues and "easily

split-off" iron. Dominici (16) has stated his belief that serum iron, iron adsorbed to red cells, and the iron present in leukocytes, all constitute iron in the process of transportation by the blood stream.

The statement has been made in the first paper of this series (17) that according to current opinion, at least three different forms of blood iron must be considered: hemoglobin iron, plasma or serum iron, and "easily split-off" iron. The physiological function and the chemical nature of hemoglobin iron are well established; but for neither of the other two is this true (see Figure 1). That the small amount of iron present as such in normal serum (50 to 180 micrograms per cent for humans) is not dialyzable, and, therefore, is not in an ionized state, is known. Nothing else is certain. It is probably trivalent, is most likely in organic combination, and is present possibly as a complex ion. The two chief views relating to its function have been intimated in the preceding paragraph. The case for "easily split-off" iron is even more perplexing. It is so termed because it is readily separated by the action of dilute acids and bases from its lightly bound state within or in association with the erythrocytes. Different concentrations of hydrochloric acid, however, "split-off" different amounts of iron (17), and both sulfuric and nitric acids "split-off" considerably greater quantities than does hydrochloric acid. Barkan and Berger observed (18) that saturation of blood with carbon monoxide "bound" about 60 to 70 per cent of the total "easily split-off" iron fraction in such a way as to protect it from the dissociating action of hydrochloric acid. Barkan (19) has presented considerable experimental evidence tending to show that "easily split-off" iron is an organic, non-hemoglobinous form of iron. He is of the opinion that it is intimately linked with the function of iron transport.

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<sup>2</sup> These studies were begun during the tenure of a National Research Council Fellowship in the Department of Medical and Surgical Research, Ohio State University, and continued during the holding of an Eli Lilly Fellowship.

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glutamate, and ferric pyrophosphate have been used in varying dosage. Blood samples were withdrawn for analysis before the ingestion of iron and at 1, 2, 4, 6, 9, 12, and 24 hours thereafter. Increases in the serum iron concentration to from three to ten times the basal level were usually observed, while the minimal change noted in "easily split-off" iron occurred as a reflection

of the serum iron increase. Data from a representative experiment are recorded in Figure 2. The subject in this instance was a 28 year old "normal" white male. Ten grams of iron and ammonium citrate were given and within 2 hours the serum iron had risen from its initial level of 100 micrograms per cent to 285 micrograms per cent. At 4 hours, the peak of 365 micrograms

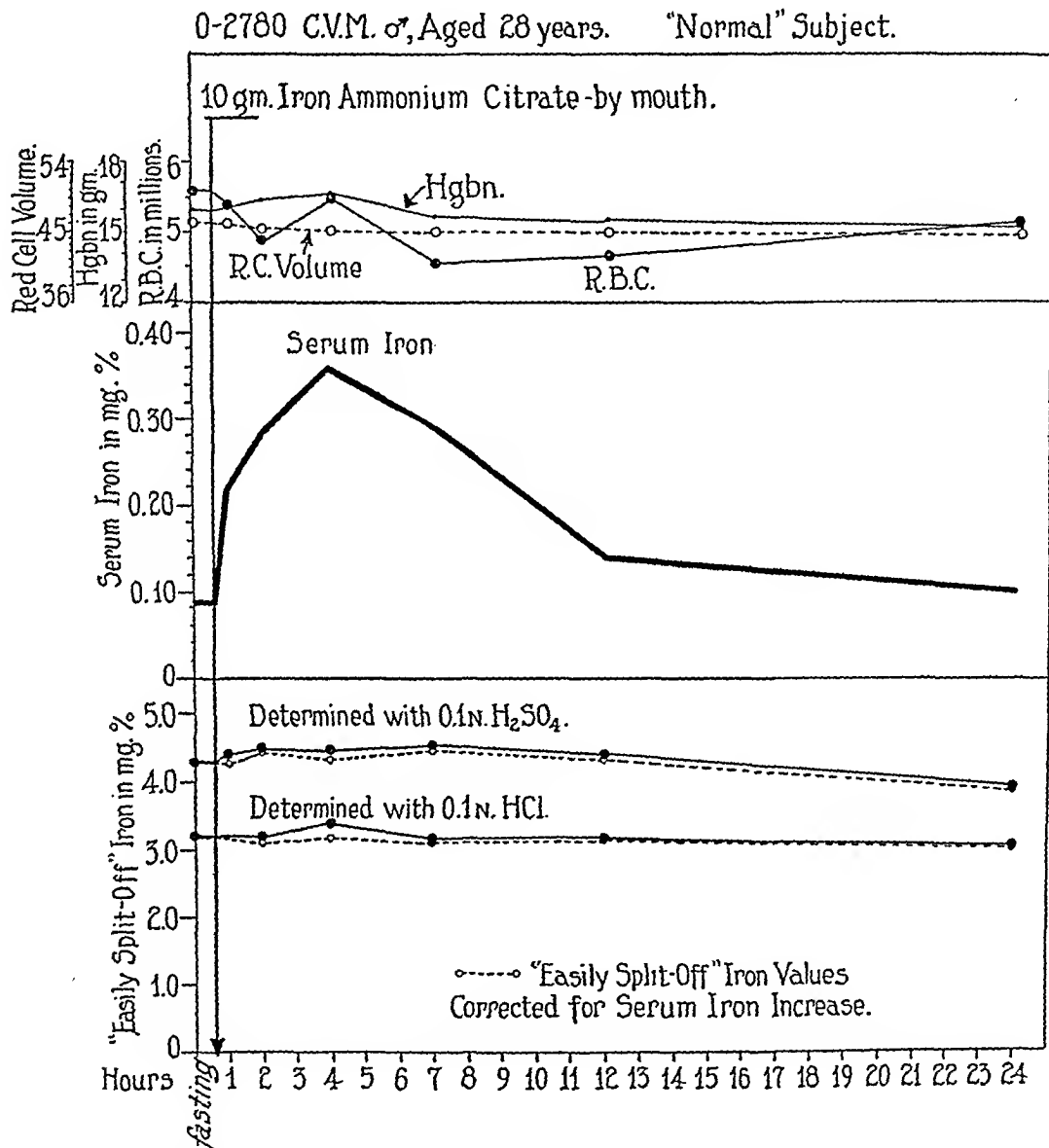


FIG. 2. SERUM IRON AND "EASILY SPLIT-OFF" IRON CURVES DURING A TWENTY-FOUR HOUR PERIOD FOLLOWING THE INGESTION OF 10 GRAMS OF IRON AND AMMONIUM CITRATE

The iron absorption is clearly reflected in the serum iron fraction. The only increase in "easily split-off" iron occurred as a result of the serum iron increase (note corrected values).

RECOGNIZED FORMS OF BLOOD IRON	NORMAL VALUES	PHYSIOLOGIC FUNCTION	CHEMICAL NATURE
1. HEMOGLOBIN IRON	Approx. 50 mg. %	Inseparably linked with the function of Hemoglobin.	Part of the Hemoglobin molecule.
2. PLASMA OR SERUM IRON.	50 - 180 $\mu\text{g}\%$	Not previously defined. (The data presented in this communication summarize the experimental evidence which tends to ascribe to plasma iron the function of iron transportation)	a) Not dialyzable, unless changed as by acidification; therefore, not in an ionized state. b) Valence: probably trivalent. c) Probably an organic form "lightly bound" in a complex radical. d) Thought by Starkenstein and his co-workers (17) to be combined with serum globulin.
3. "EASILY SPLIT-OFF" IRON	<div style="display: flex; align-items: center;"> <div style="margin-right: 10px;">           Barkan's method of ultrafiltration 1.2-2.25 mg. %            Method of dialysis used in studies (17) 1.5-3.15 mg. %         </div> <div style="border-left: 1px solid black; padding-left: 10px;">           2.9 - 4.8 mg. %         </div> </div>	Not known. Thought by Barkan to be transport iron.	a) Not dialyzable except after acidification or alkalization. b) Probably an organic form. c) Non-hemoglobinous character not definitely proved. Thought by Lintzel to be dissociated from the hemoglobin molecule, by Barkan to be non-hemoglobinous iron. d) Since "easily split-off iron and hemoglobin react similarly to adsorption on the argillaceous earths and to kataphoresis, and since both combine very readily with carbon monoxide, Barkan and Schales (17) suggest that the two molecules are probably closely related structurally.

\*  $\mu\text{g}$  = micrograms.

FIG. 1

Lintzel (20, *a* and *b*) on the other hand, has repeatedly been able to "split-off" equivalent amounts of iron from preparations of crystalline hemoglobin, and so has come to feel that "easily split-off" iron is dissociated from the hemoglobin molecule itself. In spite of the confused state of our knowledge concerning the source, chemical nature, physiological interrelationships, and actual identity of "easily split-off" iron, there can be no doubt that with the methods discussed in the preceding paper (17) a definite fraction of the blood iron, constant for any individual blood specimen, can be dissociated and accurately determined.

Since there are two forms of blood iron (serum iron and "easily split-off" iron) for which physiological functions have not been established definitely, it is logical to assume that, as, or in association with, one of them the transportation of iron in the blood stream may be effected. The present investigation represents an attempt to test the correctness of this assumption and to identify transport iron by following the responses both of the plasma and of the "easily split-off" iron values under the following conditions: 1, following various exogenous and endogenous stimuli which influence the rate of absorption of iron from the gastro-intestinal tract; 2, during varia-

tions in the rate of iron utilization by the bone marrow in its synthesis of hemoglobin; 3, with varying adequacy of the iron reserves in the body; and 4, in the presence of red cell destruction.

#### *Effect of the oral administration of iron salts on plasma and "easily split-off" blood iron*

Thoenes and Aschaffenburg (12) and Bing, Hanzal, and Myers (21) have reported increases in serum iron following single doses by mouth of various of the iron salts. Marlow and Taylor (5) failed to observe any change after the administration of six grams of iron and ammonium citrate, but the normal plasma iron values (0.4 to 0.7 mgm. per cent) obtained by these workers were several times higher than those reported by the majority of investigators (17) and were as high as those obtained at the very peak of the absorptive phase in the experiments of Thoenes and Aschaffenburg and of Bing, Hanzal, and Myers.

Using the methods previously reported (17), we have followed both the serum and "easily split-off" iron values for 24 hours after the oral administration in selected individuals of a single dose of iron. Reduced iron, ferrous ammonium sulphate, iron and ammonium citrate, ferrous

TABLE I

*Serum iron and "Easily split-off" iron in clinical states of varied etiology*

Diagnosis	Subject	Age	Hematological data			Serum iron	"Easily split-off" iron	
			R.B.C.	Hb	Red cell volume		Determined with	
							0.1 N HCl	0.1 N H <sub>2</sub> SO <sub>4</sub>
		years	millions	grams	per cent	micro-grams per cent	mgm. per cent	mgm. per cent
I. Iron deficiency (hypochromic) anemias								
A. Chronic hypochromic microcytic anemia (without hemorrhage).....	L. R. ♀	24	4.09	8.7	33.5	28	1.699	
Chronic hypochromic microcytic anemia.....	J. W. ♀	46	5.05	8.9	32.5	34	1.683	2.970
Chronic hypochromic microcytic anemia.....	H. S. ♀	49	3.72	5.8	25.0	19	1.136	2.149
Chronic hypochromic microcytic anemia.....	E. B. ♀	23	5.41	10.1	31.0	46		
Chronic hypochromic microcytic anemia.....	J. S. ♂	36	1.92	3.2	13.0	22		
Chronic hypochromic microcytic anemia.....	H. B. ♀	28	2.94	5.7	22.5	28	1.321	2.027
B. Following chronic hemorrhage.....	M. N. ♀	42	2.63	4.0	12.0	23	0.855	1.505
Following chronic hemorrhage.....	E. S. ♂	49	1.47	2.2	11.5	17		
Following chronic hemorrhage.....	L. Van W. ♀	55	2.52	4.4	14.0	14		1.487
Following chronic hemorrhage.....	J. B. ♀	38	4.01	7.1		29		
Following chronic hemorrhage.....	H. E. ♂	44	5.35	9.8	33.0	32		4.580
C. Following therapeutic phlebotomy in polycythemia vera.....	F. E. ♀	47	6.78	15.6	56.0	22	2.148	3.947
Following therapeutic phlebotomy in polycythemia vera.....	A. K. ♂	43	6.82	14.8	48.5	37	2.076	3.926
Following therapeutic phlebotomy in polycythemia vera.....	E. S. ♂	46	4.62	11.4	42.8	46	1.936	3.650
II. Acute hemorrhage								
A. From peptic ulcer, 4 days following hemorrhage.....	J. P. ♂	45	1.400	5.0	15.0	71		
48 hours later.....			1.570	5.5		35		
B. Epistaxis, within 24 hours following.....	J. D. ♀	60	3.36	6.3	23.6	97	2.010	2.719
1 week later.....			2.86	5.9	22.0	31		2.347
III. Hypoplastic anemia, idiopathic								
Hypoplastic anemia.....	D. L. ♂	25	2.79	8.9	26.0	209		
"Primary" aplastic anemia.....	O. E. ♂	53	0.91	3.4	10.5	294		
"Primary" aplastic anemia after transfusion.....			1.94	7.8	24.0	165	1.534	
"Primary" aplastic anemia after remission.....			3.73	12.7	42.0	86	2.864	4.310
"Primary" aplastic anemia.....	C. C. ♂	47	1.29	5.0		313	1.175	
			1.06	4.6		296		1.534
			2.00	6.2		282	1.577	2.610
IV. Myelophthisic anemia								
Accompanying aleukemic leukemia (myelogenous).....	Dr. S. ♂	57	2.01	7.3	16.0	205		
Accompanying aleukemic leukemia (lymphatic).....	P. R. ♀	40	1.73	6.6	23.0	158	1.540	3.125
V. Macrocytic, hyperchromic anemia (untreated)								
Addisonian pernicious anemia.....	S. J. ♂	41	1.57	6.0	16.5	348	1.803	
Addisonian pernicious anemia.....	H. M. ♂	63	1.43	6.4	23.0	194	2.404	
Addisonian pernicious anemia.....	A. B. ♀	54	2.49	11.6	32.0	154	2.048	3.159
Addisonian pernicious anemia.....	W. W. ♀	42	1.82	6.7	20.5	167	2.424	
Addisonian pernicious anemia.....	S. B. ♂	77	1.41	5.4	19.0	211	1.456	2.575
Addisonian pernicious anemia.....	P. B. ♀	36	0.79	3.0	8.3	295		2.510
Addisonian pernicious anemia.....	W. S. ♂	65	0.67	3.2	9.5	220	1.835	
Addisonian pernicious anemia.....	L. R. ♂	49	1.47	6.0	15.0	306		
Pernicious anemia of pregnancy.....	G. H. ♀	22	1.48	6.6	18.0	270	1.773	2.655
Dietary deficiency of extrinsic factor.....	V. M. ♀	53	1.57	7.1	20.0	147		
Pernicious anemia accompanying carcinoma of stomach.....	J. S. ♂	59	1.34	5.1	21.0	278	2.026	2.727
VI. Congenital hemolytic icterus								
A. Acute hemoclastic crisis	B. Y. ♀	57						
Before splenectomy.....			1.43	4.8	16.0	157		
15 minutes after splenectomy.....			2.270	6.5	25.0	118		
8 hours after splenectomy.....			2.320	6.3	25.5	61		
B. Subacute phase								
Before splenectomy.....	B. B. ♀	26	2.58	7.2	21.7	160	2.432	3.970
15 minutes after splenectomy.....			3.41	11.0	33.0	80	2.575	4.180
7 hours after splenectomy.....			3.18	10.9	32.0	56		



per cent was reached. When correction was made for this serum iron response, it was evident that no significant change had occurred in the "easily split-off" iron fraction. In general, the rise in serum iron usually began within the first half hour, reached its maximum at the end of 2 to 5 hours, and then fell gradually to reach the basal level at the end of 8 to 12 hours. The highest values obtained were in the neighborhood of 600 micrograms per cent.

Significant increases in serum iron were observed following iron and ammonium citrate in single doses varying from 2 to 20 grams. One normal subject (V. C., a young woman aged 26 years with normal gastric acidity) failed to show any serum iron response to 0.5 gram of reduced iron, but had a substantial rise (128 to 345 micrograms per cent) after 5 grams. A series of observations were made on Patient E. B., a 25 year old nurse in whom a diagnosis of hypochromic microcytic anemia with achlorhydria (histamine refractory) had been made. Two years before the studies here referred to were begun, the patient had responded to adequate iron therapy with a typical reticulocyte response and rise in hemoglobin. Upon discontinuing therapy, however, the hemoglobin gradually fell to the original low level again. Five grams of reduced iron were given (October 13, 1934), and the serum iron rose from its base line level of 45 micrograms per cent to a peak of 255 micrograms per cent at the end of 5 hours. Two weeks later (October 31, 1934), without any intervening therapy having been given, the study was repeated except that 50 cc. 1 N HCl were given with the 5 grams of reduced iron. At this time, the serum iron rose from approximately the same initial level to a high of 400 micrograms per cent at the end of two and one-half hours, and this high level was maintained for at least four additional hours.

Measurements of the blood iron changes following the oral administration of iron salts have been made on more than twenty subjects, and among these only three have failed to show increases in the plasma or serum iron concentration. One of the exceptions was a middle aged white male acutely ill with a rare mycotic infection. The second was a 55 year old retired physician who was, at the time of the observation, in an aleukemic phase of chronic myelogenous leukemia.

Both of these men received 15 grams of iron and ammonium citrate in one dose; both had normal gastric acidity. The third patient was a 38 year old colored female with achylia gastrica and typical pernicious anemia in relapse. She failed to show an increase in her serum iron level after 4 grams of reduced iron alone, and also after 4 grams of reduced iron given with 30 cc. 1 N HCl. With the reduced iron increased to 6 grams and acidified, however, a 50 per cent increase over the basal serum iron value did occur. All three of these patients were acutely ill at the time the observations were made. Any reason otherwise for their failure to respond, as did the rest of the subjects, is not at present apparent.

The observation that, following the oral administration of iron salts, serum iron is the only iron fraction to increase significantly in the blood during the phase of intestinal absorption, is in agreement with certain *in vitro* observations of Starkenstein and Weden (14) and of Barkan (15, a; 22). These workers added graded amounts of various of the iron salts to whole blood *in vitro* and found that invariably all of the added iron could subsequently be recovered from the plasma; no increase in iron occurred in association with the cells. Barkan (22) further noted that when iron salts were added to plasma or whole blood outside of the body, the iron was changed so that it would no longer pass through a semi-permeable membrane—unless acidified. Similarly, in our experiments, the increased iron in the plasma presumably the result of absorption from the gastro-intestinal tract was in an unionized state and could not be dialyzed through a cellophane membrane.

#### *The iron fractions of the blood in iron deficiency states*

In the iron deficiency anemias of varied etiology, we have found the serum iron values (see Table I) to be definitely lower (15 to 40 micrograms per cent) than the zonal range for normal. Similar results have been obtained by Locke, Main and Rosbash (11) in rabbits made anemic by repeated hemorrhages and in a few patients with iron deficiency anemias. Thoenes and Aschaffenburg (12) report low serum iron values in nutritional deficiency in children. Barkan (23) had opportunity to study the plasma iron level in

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A. Acute hemoclastic crisis								
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15 minutes after splenectomy.....			3.41	11.0	33.0	80	2.575	4.180
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TABLE I—Continued

Diagnosis	Subject	Age	Hematological data			Se- rum iron	"Easily split- off" iron	
			R.B.C.	Hb	Red cell volume		Determined with	
							0.1 N HCl	0.1 N H <sub>2</sub> SO <sub>4</sub>
		years	millions	grams .	per cent	micro- grams per cent	mgm. per cent	mgm. per cent
VII. Miscellaneous								
Malignant neutropenia.....	J. F. ♀	28	4.38	12.5	40.0	66		
Malignant neutropenia.....	C. C. ♀	34	3.75	11.9	38.0	88	2.115	
Monocytic leukemia.....	J. S. ♂	10	1.82	6.1		228	1.375	2.097
Myelogenous leukemia.....	R. W. ♂	26				73		
Leukosarcoma.....	E. J. ♀	6	2.06	5.4	16.0	114		
Polycythemia vera (untreated).....	F. E. ♀	47	10.31	30.3	77.0	90	3.238	5.941
Polycythemia vera (untreated).....	E. S. ♂	46	9.84	17.0	64.0	46	2.085	
Multiple myeloma.....	E. S. ♀	52	3.92	10.7	39.0	60		3.000
Hemophilia.....	L. W. ♂	11	2.59	8.8	23.5	116		
Thrombocytopenic purpura.....	E. M. ♀	18	4.44	12.1	38.0	172		
Tuberculosis—Sickle cell trait.....	L. R. ♂	17	3.44	8.4	27.3	65	1.905	2.448
Carcinoma of prostate.....	S. N. ♂	64	2.400	8.0	25.3	84		3.030
Abscess of prostate and of left thigh.....	O. Van W. ♂	52	1.26	4.4	16.0	56	1.147	1.986
"Banti's disease".....	W. W. ♀	38	2.65	6.2	23.0	19	2.336	2.924
✓ Acute yellow atrophy of liver.....	E. L. ♀	40	3.58	10.6	31.0	202		
✓ Cirrhosis of liver (portal).....	D. S. ♂	45	3.75	10.7	33.8	66		
✓ Hemochromatosis.....	M. W. ♀	64	4.02	11.8	35.0	101	2.419	4.480
Chronic nephritis, arteriolar sclerotic.....	C. K. ♀	45	3.05	7.9	26.0	54	1.924	2.978
Multiple nutritional deficiency.....	A. S. ♀	33	2.84	10.2	29.7	56	2.020	3.333
Diabetes mellitus treated.....	E. F. ♂	28	4.90	18.1	41.0	152		
Hypothyroidism.....	M. H. ♀	26	4.30	12.1	40.1	165	3.125	4.545
Hypothyroidism.....	C. A. ♀	42	4.13	10.7	33.0	140	2.607	4.199

horses from the Serum Institute of Dorpot in whom anemias had developed following frequent bleedings over a long period. The plasma iron values in these animals were higher, not lower, than normal. What complicating factors, if any, such as liver or marrow damage, may have been present in these "serum-producing" animals to alter further their iron metabolism and thus cause the plasma iron levels to be high, are, of course, not known.

The "easily split-off" iron values, on the other hand, showed, in our series of cases, no consistent change. High normal as well as low and lower than normal figures were obtained. Bar-kan (15, a) observed that the oral administration of iron to rabbits for one to two weeks failed, with one exception, to cause any increase in the "easily split-off" blood iron. The exception occurred in the case of an animal whose initial "easily split-off" iron level was lower than the average (0.840 mgm. per cent); an increase to 1.400 mgm. per cent was obtained.

In order that the blood iron relationships in human iron deficiency states might be understood more completely than is possible from isolated single determinations in different patients, serial studies of serum and "easily split-off" iron were made in a number of selected individuals with hypochromic anemia over extended periods with and without iron therapy. The data from two representative cases is summarized in Figures 3 and 4. The first of these (Figure 3) presents the observations made on E. R., a young woman, 25 years old, with hypochromic microcytic anemia and hypochlorhydria. Following the oral administration of 6 grams of reduced iron in one dose, her serum iron rose from its initial low level of 40 micrograms per cent to a peak value slightly over 500 micrograms per cent. After this initial observation, the patient was placed on a daily dose of 4 grams of iron pyrophosphate. A reticulo-cyte response (to 6 per cent) beginning on the sixth day of therapy was observed, and the hemoglobin gradually rose from its initial level of 9 grams per 100 cc. to 13 grams by the end of two

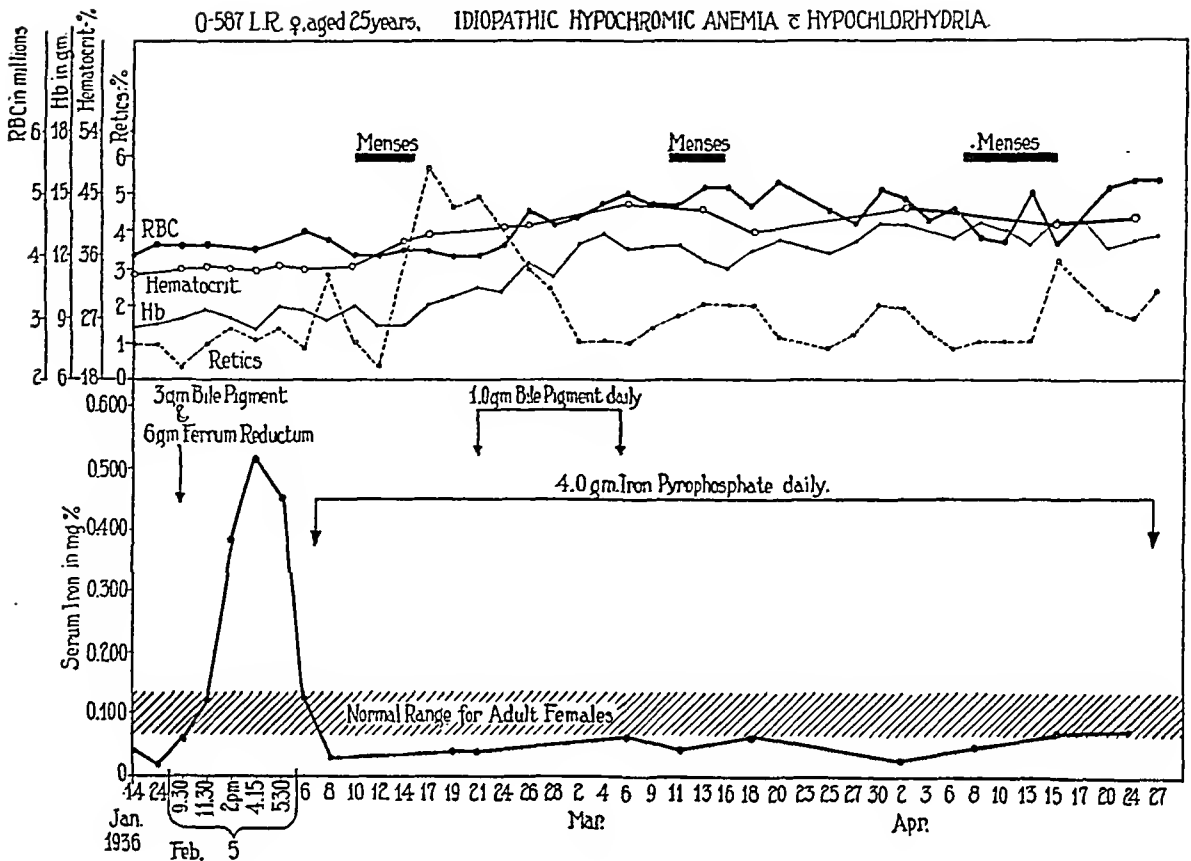


FIG. 3. OBSERVATIONS ON THE SERUM IRON LEVEL IN A PATIENT WITH HYPOCHROMIC MICROCYTIC ANEMIA DURING A PERIOD OF INTENSIVE IRON THERAPY

As an initial observation, a substantial rise in serum iron during the phase of intestinal absorption following a single large dose of ferrum reductum was demonstrated. The postabsorptive level of serum iron, however, did not increase to the normal range until after the hemoglobin had approximated the normal.

and one-half months. The serum iron,<sup>3</sup> meanwhile, slowly increased from a basal value of 30 to 40 micrograms per cent to 60 to 70 micrograms per cent, the latter being within the normal zonal range. "Easily split-off" iron changed only from 1.7 to 2.0 mgm. per cent (as determined with 0.1 N HCl), both of which figures are within the limits established for normal.

The second experiment to be presented in detail (Figure 4) was performed on H. S., a 45 year old woman with hypochromic microcytic anemia and an histamine refractory achlorhydria. This patient was given 1.2 grams ferrous sulphate

in capsules daily during the early period of study, and 6 grams of iron and ammonium citrate daily during the last six weeks of observation. A reticulocytosis (to 10 per cent) developed following the institution of iron therapy, and the hemoglobin rose from 6 to 12 grams per cent. The basal "easily split-off" iron values were approximately 2.0 mgm. per cent (as determined with 0.1 N H<sub>2</sub>SO<sub>4</sub>), that is, below normal. Blood for iron analyses<sup>3</sup> was taken at weekly intervals. The "easily split-off" blood iron was still approximately 2.0 mgm. per cent in the specimen taken six days after the commencement of iron therapy, but by the end of the second week, it had practically doubled in value. No significant change from this higher figure occurred throughout the rest of the experiment. This in-

<sup>3</sup> Whenever blood was to be withdrawn for iron analyses, the iron dosage was omitted during the 24 hour period prior to venepuncture.

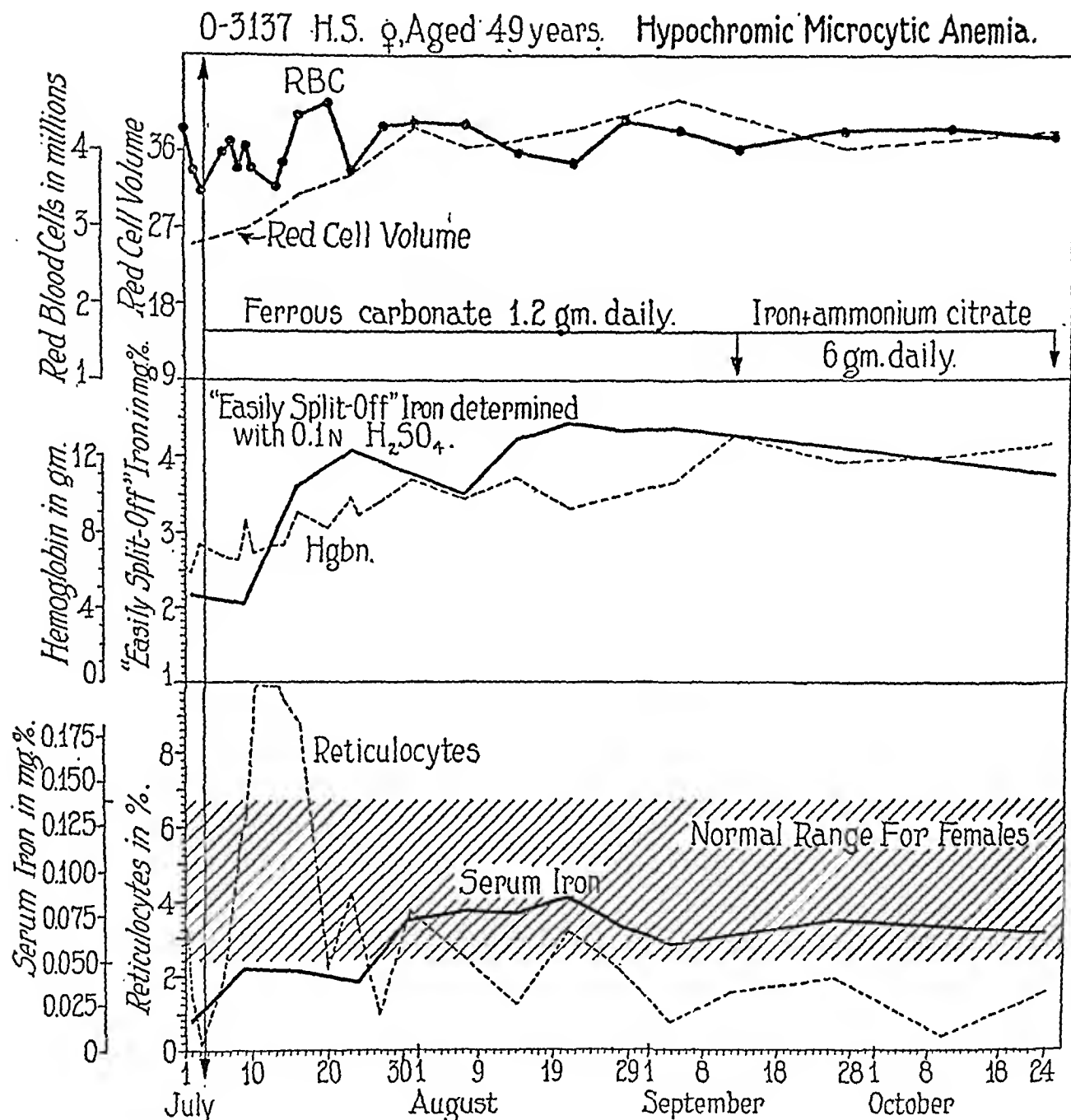


FIG. 4. BLOOD IRON RELATIONSHIPS IN A PATIENT WITH HYPOCHROMIC, MICROCYTIC ANEMIA DURING A PERIOD OF INTENSIVE IRON THERAPY

The "easily split-off" iron increased to the normal zonal range before any substantial rise in hemoglobin had occurred. Serum iron rose to normal only after the hypochromia of the red cells had been corrected.

crease was in line with the previously mentioned observation by Barkan (15, a) that animals with initially low "easily split-off" blood iron values may show a rise to normal under the influence of oral iron medication, whereas no change occurred in those in whom the level initially was within normal limits. The serum iron curve, however, proceeded quite independently and reached the normal zonal range for females only after an ad-

ditional two weeks of therapy—at a time when both the mean corpuscular volume and the mean corpuscular hemoglobin concentration had likewise become normal. In other words, the plasma or serum iron did not return to normal until after the hypochromia of the red cells had disappeared, the iron deficiency state had been corrected, and the bone marrow had settled down to a more normal state of erythrocytogenesis.

Briefly, in the human iron deficiency states, plasma iron was found always to be low, while "easily split-off" iron was present in diminished to normal quantities. Indeed, in the moderate hypochromic microcytic anemias produced in three patients with polycythemia vera by therapeutic bleeding over a long period of time, the "easily split-off" iron values were frequently in the higher portion of the normal zonal range. Under the influence of iron therapy, plasma iron values returned to normal as the mean corpuscular hemoglobin concentration increased to normal and as the state of iron deficiency was corrected. When the initial "easily split-off" blood iron level was low, a return to the average was effected by iron therapy; otherwise, no change occurred.

#### *Acute hemorrhage*

The opportunity was afforded us on one occasion to observe the serum iron level in a middle-aged physician several days following massive hemorrhage from a previously "silent" duodenal ulcer. At that time, the serum contained 71 micrograms per cent of iron. Two days later, however, the value had fallen to 35 micrograms per cent. This stimulated us to follow the blood iron relationships under the influence of phlebotomy performed as a therapeutic measure in the treatment of patients with polycythemia vera. The data from one such set of observations, made on E. E., a 47 year old white woman with previously untreated polycythemia vera, are recorded in Figure 5. During a four day period, 3450 cc. of blood were removed. The red cells fell from 10 to 6 million, the hemoglobin from 31 to 15 grams, and the hematocrit reading from 78 to 48 per cent. The serum iron level oscillated from 50 to 80 micrograms per cent for the first three days and then fell sharply to the low figure of 18 micrograms per cent. Determinations made during the subsequent two months showed a slight rise in the serum iron curve, but the lower limits of the normal zonal range were not reached. Relatively, a much smaller decrease in "easily split-off" iron (from 6.0 to 5.0 mgm. per cent) was noted during and immediately following this period of induced hemorrhages. The iron fraction continued its downward trend, however, throughout the rest of the period, and had fallen to 3.95 mgm. per cent at the end of that time.

In brief, within 48 to 96 hours following an acute hemorrhage, a fall in serum iron occurred to either a low normal or a lower than normal level. This change occurred at approximately the same time that the compensatory hyperplasia of erythroid elements in the bone marrow probably became evident. "Easily split-off" iron, however, showed no striking change at the time of hemorrhage, but fell slowly over a period of weeks thereafter. There was distinctly no correlation between the hemoglobin and the "easily split-off" iron curves.

#### *Hypoplastic anemia*

The hypoplastic anemias have been of special interest in our attempt to locate and identify transport iron in the blood because, when uncomplicated by hemorrhage, there should be no deficiency of iron, and because an hypoplastic bone marrow obviously will utilize only small amounts of iron in hemoglobin synthesis. The concentration of transport iron in the blood stream should, therefore, tend to be high if the storage depots retain their ability to supply iron to the blood while the bone marrow becomes progressively less able to utilize it.

Serum iron in the five cases of hypoplastic anemia in our series was found to vary from a high normal value of 158 micrograms per cent upward to the abnormally high value of 313 micrograms per cent (Table I). It will be noted that the highest figures obtained for serum iron were in the two cases of apparently "primary" aplastic anemia. It is of additional interest that when a remission developed in one of these patients (O. E.), his serum iron fell from its initial level of approximately 300 micrograms per cent to a normal value of 86 micrograms per cent, reflecting directly the increase in erythropoietic activity. "Easily split-off" iron was determined in only three cases. In two, the values were within normal limits; in the third (C. C.), they were below normal.

#### *Pernicious anemia*

In pernicious anemia, opportunity is afforded for the study of blood iron relationships in the same individual under markedly contrasting conditions. During a relapse, there is a variable amount of abnormal red cell destruction, dimin-

## POLYCYTHEMIA VERA

0-3260 F.E. ♀ Aged 47 years. Blood Iron Changes Following Therapeutic Phlebotomy.

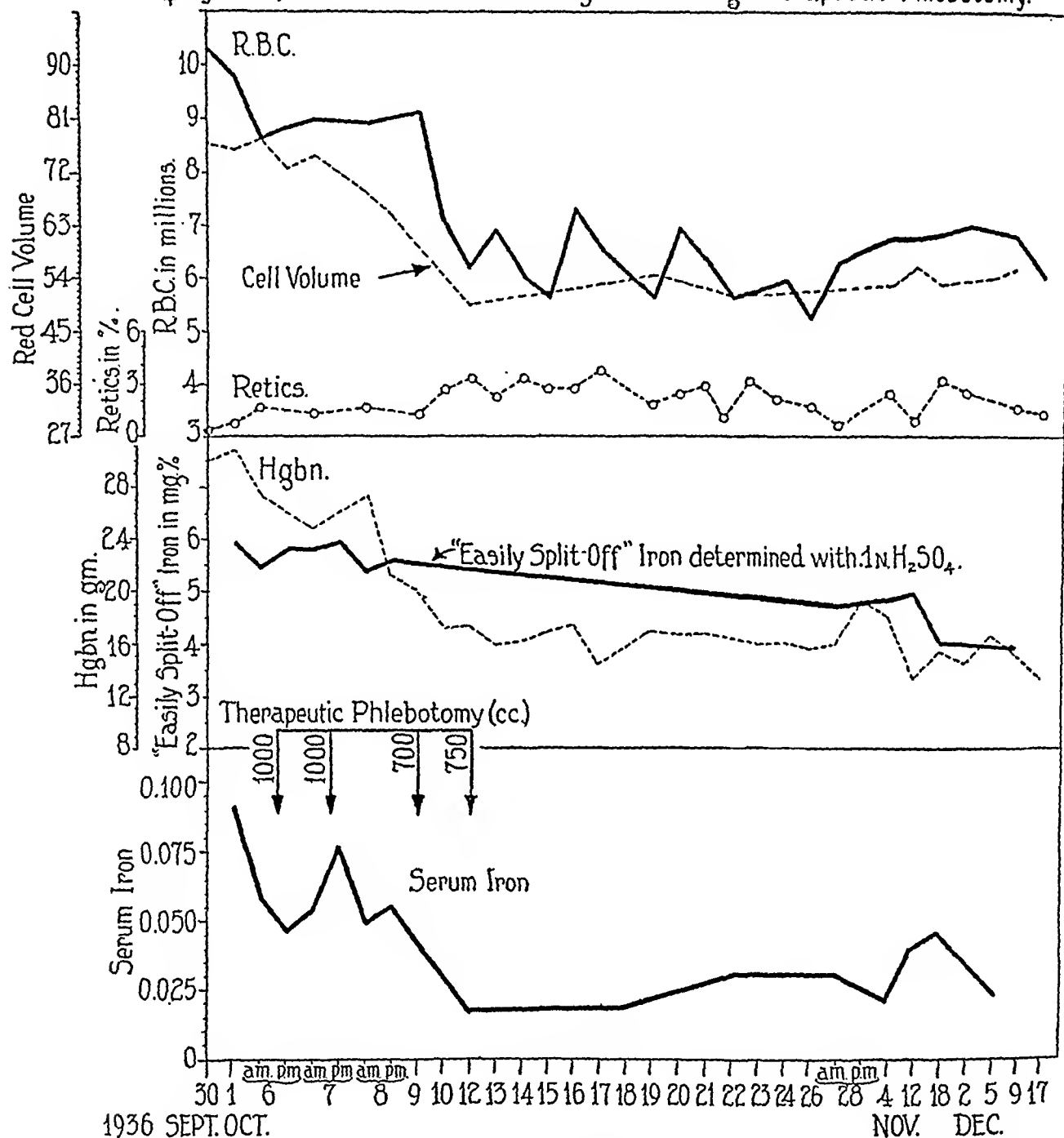


FIG. 5. EFFECT OF THERAPEUTIC PHLEBOTOMY ON THE BLOOD IRON FRACTIONS IN A PATIENT WITH POLYCYTHEMIA VERA

A sharp fall in the serum iron curve was obtained on the fourth day. The decrease in "easily split-off" iron was much more gradual and continued throughout the period of observation.

ished hemoglobin synthesis as the result of maturation arrest, but no iron deficiency usually. All of these factors would tend to elevate the level of iron being transported in the blood stream. Following the institution of liver therapy, however, there is unusually rapid hemoglobinogenesis. The bone marrow might be ex-

pected, under these circumstances, to require iron in relatively large quantities, and if this supply must be transported from the storage depots by the blood, the demand could conceivably be greater than the ability to respond. The transport iron concentration accordingly would tend to be lower than normal.

In our cases of pernicious anemia in relapse (Table I), the plasma or serum iron levels were found to be uniformly high (147 to 348 micrograms per cent) whereas the "easily split-off" iron values were within normal limits with low as well as high "normals" being recorded. The series of cases studied included ten patients with Addisonian pernicious anemia, one with a macrocytic anemia which developed as the result of a food-phobia, dietary deficiency with insufficient "extrinsic factor" (Mrs. M.), and one with pernicious anemia of pregnancy (G. H.), also dietary in origin.

Soon after liver was given and slightly prior to the reticulocyte response, a precipitous drop in the concentration of serum iron from its original high value to a lower than normal level occurred in all cases. This phenomenon is analyzed in Figure 6 which tabulates the data obtained from

S. J., a 46 year old white male with typical Addisonian pernicious anemia. Determinations of blood iron were made at eight hour intervals following one intravenous injection of liver extract derived from 100 grams of raw liver. The curve of decreasing serum iron (from approximately 300 micrograms per cent to 60 micrograms per cent in 6 days) began its downward course within the first 24 hours and reached its greatest depression at the peak of the reticulocyte response, i.e. at the time of maximum delivery of young erythrocytes from the bone marrow.

To another patient with pernicious anemia (W. S., a 65 year old white male) (Figure 7) was given a suboptimal dose of liver intramuscularly. The reticulocytes rose to a peak of 36 per cent by the sixth day, and the serum iron fell from 230 to 50 micrograms per cent. Some increase in both red cells and hemoglobin oc-

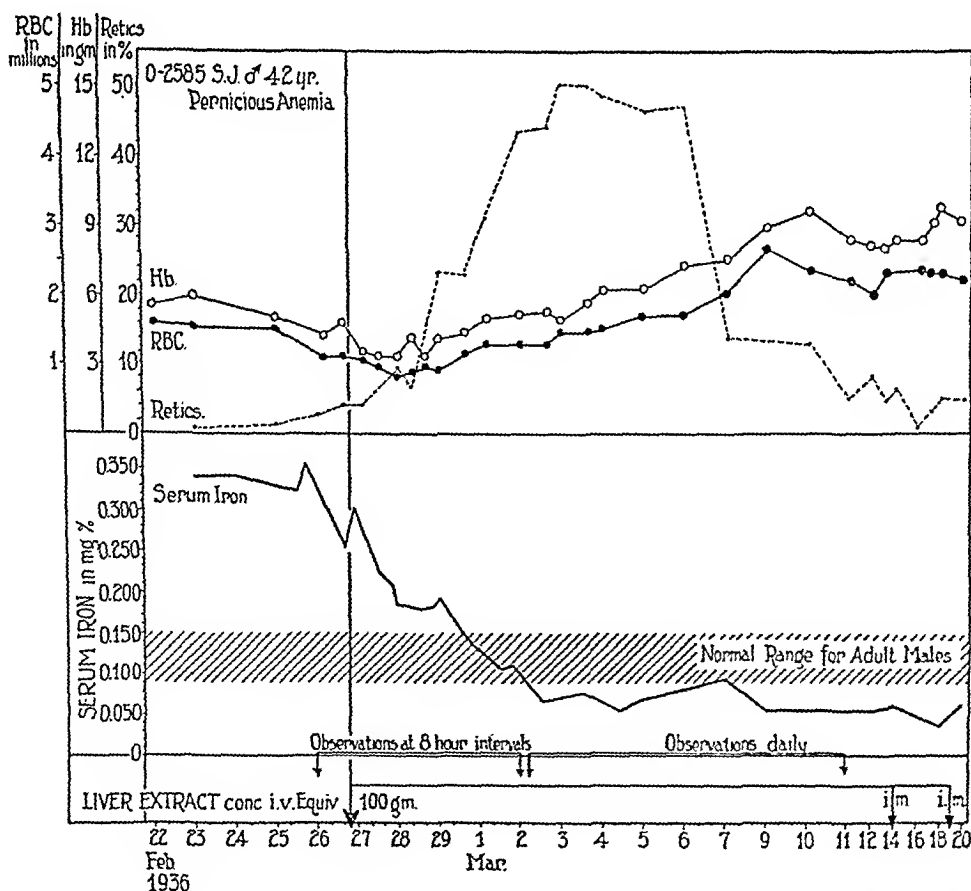


FIG. 6. EFFECT OF LIVER EXTRACT ON RETICULOCYTES AND ON SERUM IRON LEVEL.



## POLYCYTHEMIA VERA

O-3260 F.E. ♀ Aged 47 years. Blood Iron Changes Following Therapeutic Phlebotomy.

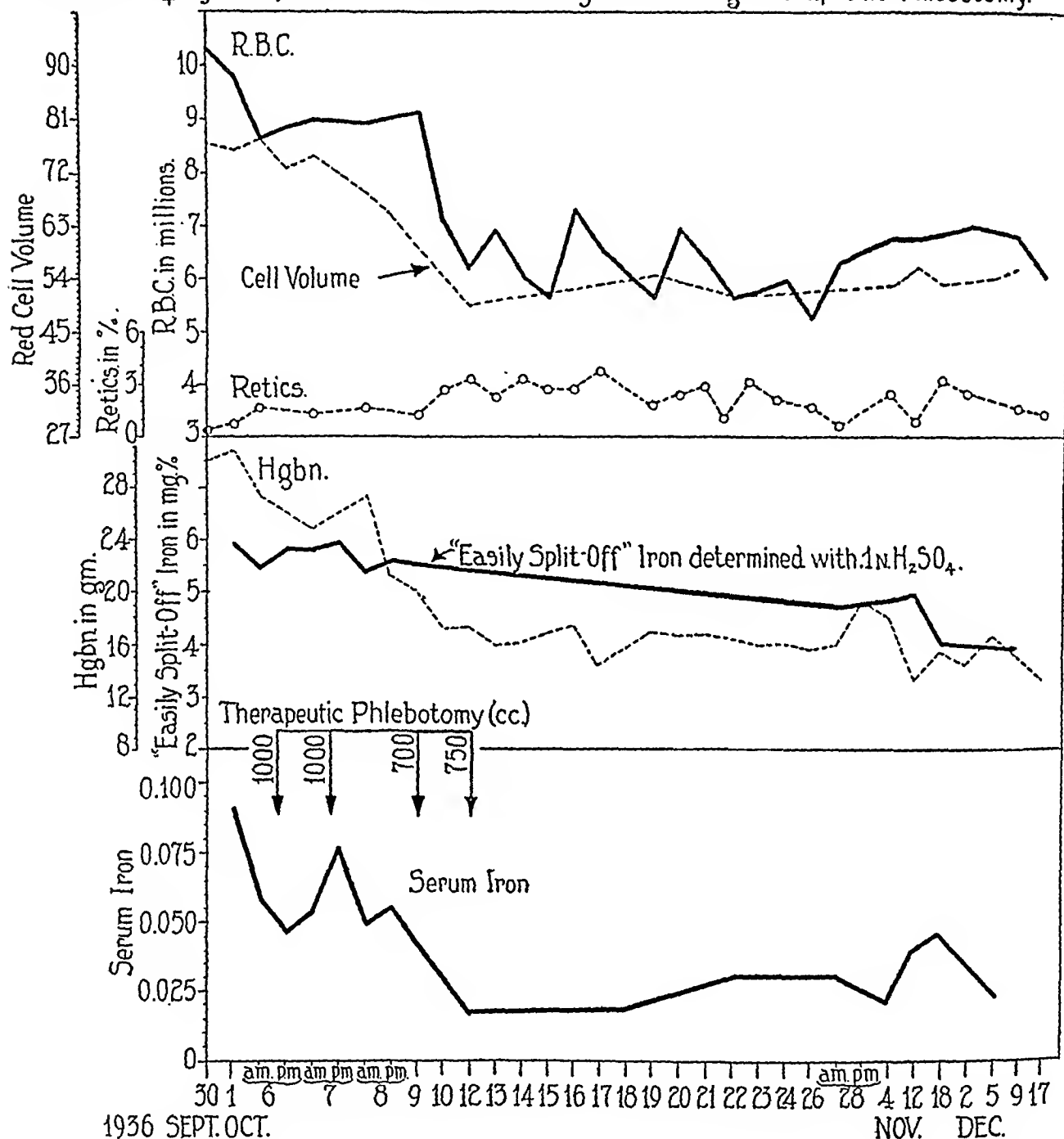


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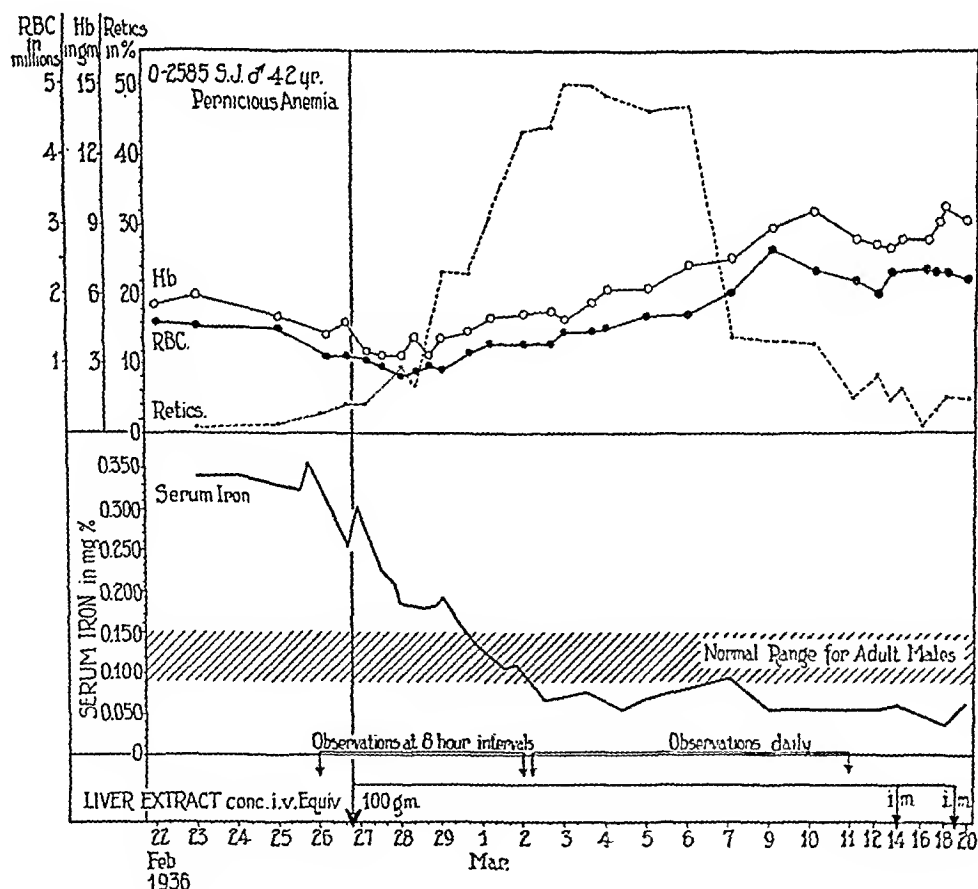


FIG. 6. EFFECT OF LIVER INDUCED REMISSION ON RETICULOCYTOSIS AND THE SERUM IRON LEVEL

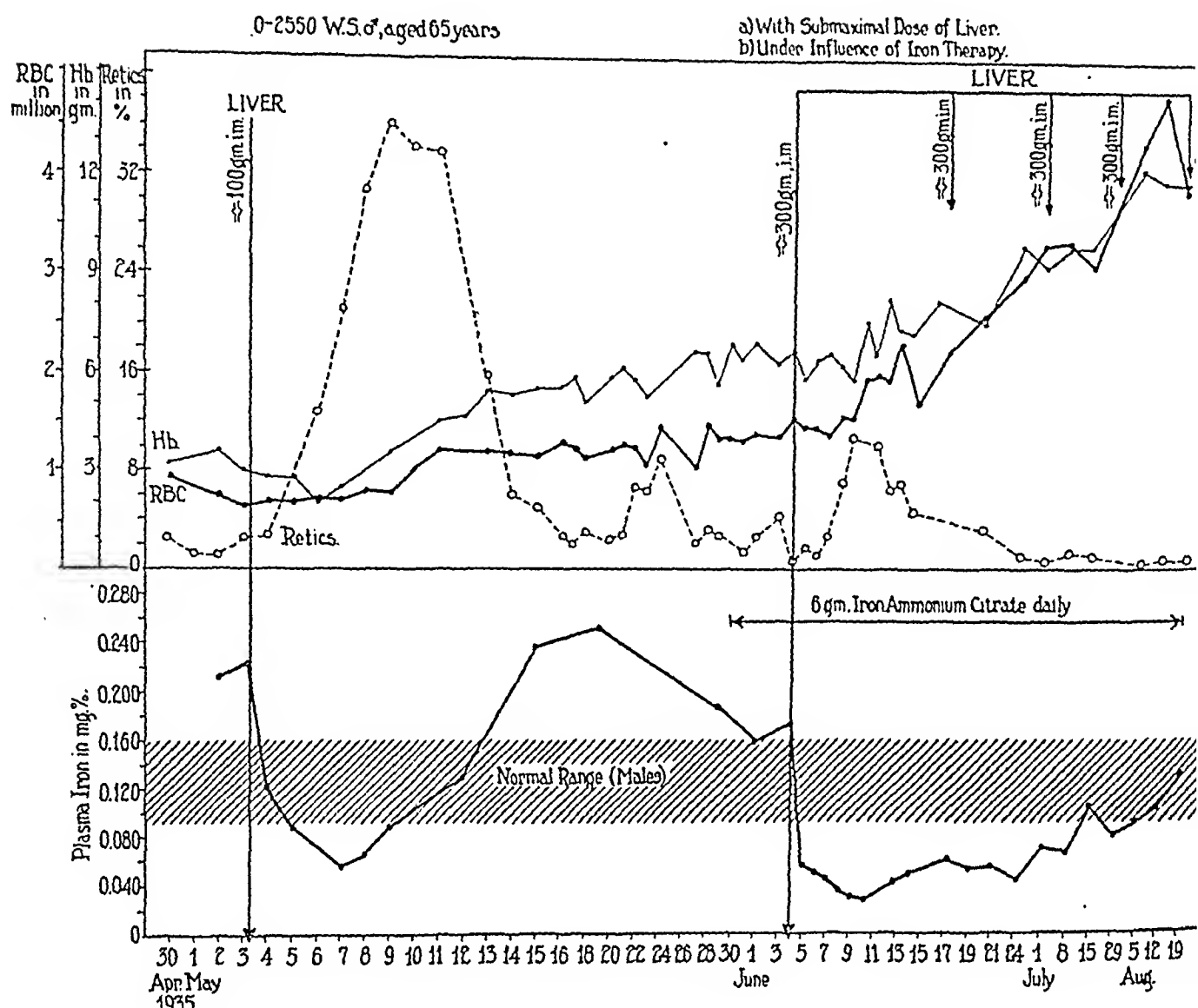
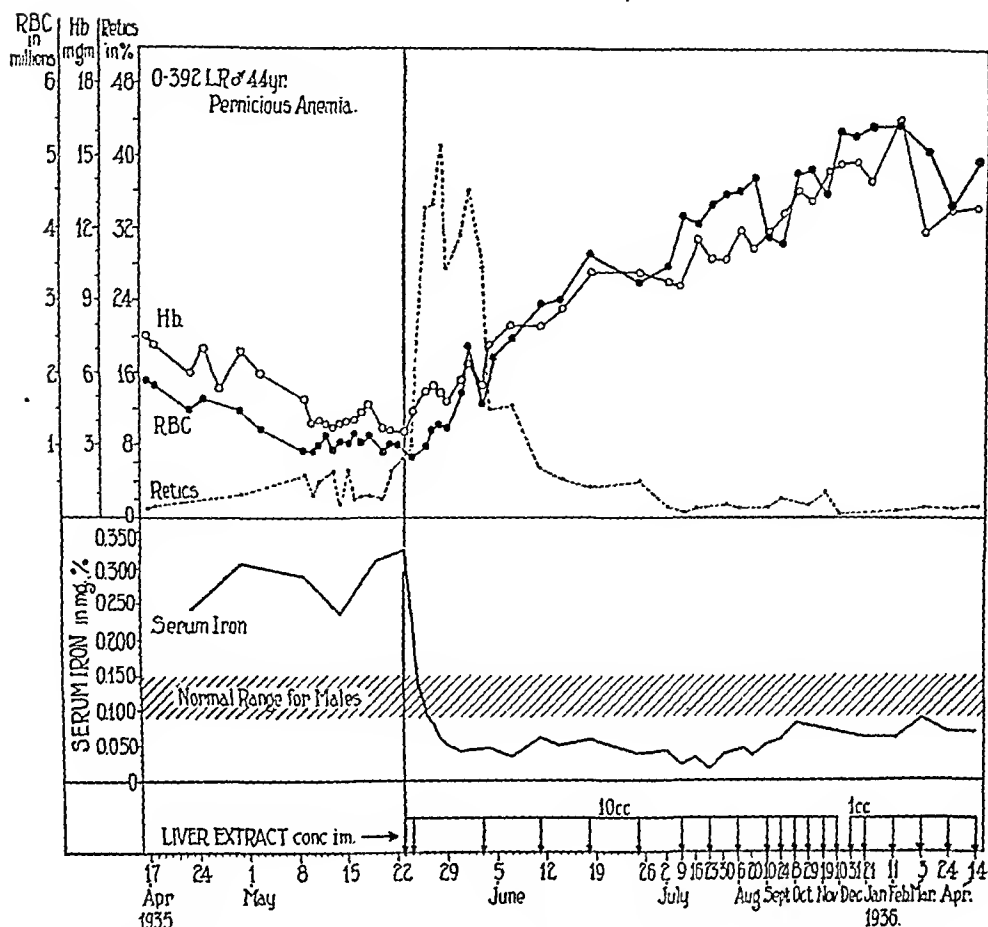


FIG. 7. EFFECT OF LIVER INDUCED REMISSIONS ON THE PLASMA IRON LEVEL IN PERNICIOUS ANEMIA

curred; but as the effect of the liver became less apparent and the erythrocytes ceased to rise, the values for serum iron rose once more to the high pre-therapy level. When the administration of liver extract in adequate amounts was reinstituted, a second reticulocyte response and reciprocal fall in serum iron was observed. During this second period, six grams of iron and ammonium citrate were given daily. Care was taken to see that the iron was administered in 2 gram doses at 12 noon, 3 p.m. and 6 p.m. Blood was then withdrawn at 11:30 a.m. each day for iron analysis—after a 17½ hour “iron fast.” Within two months, the erythrocytes rose to the four million level and the hemoglobin to 12 grams per cent. As the blood picture approached normal and the rate of erythrocytogenesis decreased, the serum iron promptly rose to enter the normal zonal range.

A third patient, L. R., a colored male, 52 years old (Figure 8), is presented in contrast to the previous case. This man was treated from the start with adequate amounts of liver parenteral. He developed a typical reticulocyte response and the characteristic precipitous fall in serum iron. His therapy was not supplemented by iron, however, and it is interesting to note that his serum iron level did not return to normal until almost a year after the beginning of the liver induced remission.

In a fourth patient, H. H. M. (Figure 9) “easily split-off” iron was determined in addition to plasma or serum iron. The irregularity of the plasma iron curve—the initial fall occurred as usual—was due to a relative refractoriness to liver therapy. It will be noted that following both the second and the third administrations of



liver, appreciable drops in serum iron were observed. The important thing in this study, by contrast, was the failure of the "easily split-off" blood iron values to show any significant changes during the entire period of observation.

G. H., the fifth case, a 22 year old white woman, presented herself during the third trimester of her fifth pregnancy with a severe anemia. The gastric acidity was normal but the diet had been grossly deficient in all essential elements including animal protein. The blood and bone marrow findings were typical of pernicious anemia with macrocytosis, megaloblastic hyperplasia, and a high serum iron. A deficiency of the "extrinsic factor" of Castle was the obvious etiological factor. When this deficiency was corrected through the institution of an adequate hospital diet supplemented by autolyzed yeast, a typical reticulocyte peak (49 per cent) was fol-

lowed by a substantial increase in red cells and hemoglobin. Serial supravital differential cell counts of sternal bone marrow obtained by the puncture technique were made (Figures 10 and 11) in order to correlate the changes in bone marrow cellular equilibria with the re-equilibrations of blood iron. A precipitous fall in serum or plasma iron began within 24 hours following the institution of adequate therapy and occurred coincident with the rapid disappearance of the original megaloblastic predominance in the bone marrow. As the level of red cell maturation shifted further to the right, it became apparent that the rapid increase in normoblasts in the marrow paralleled the reticulocyte rise in the peripheral blood. As the rate of hemoglobin synthesis became accelerated, as the megaloblasts matured, the serum iron fell precipitously to the lower limits of normal. The "easily split-off"

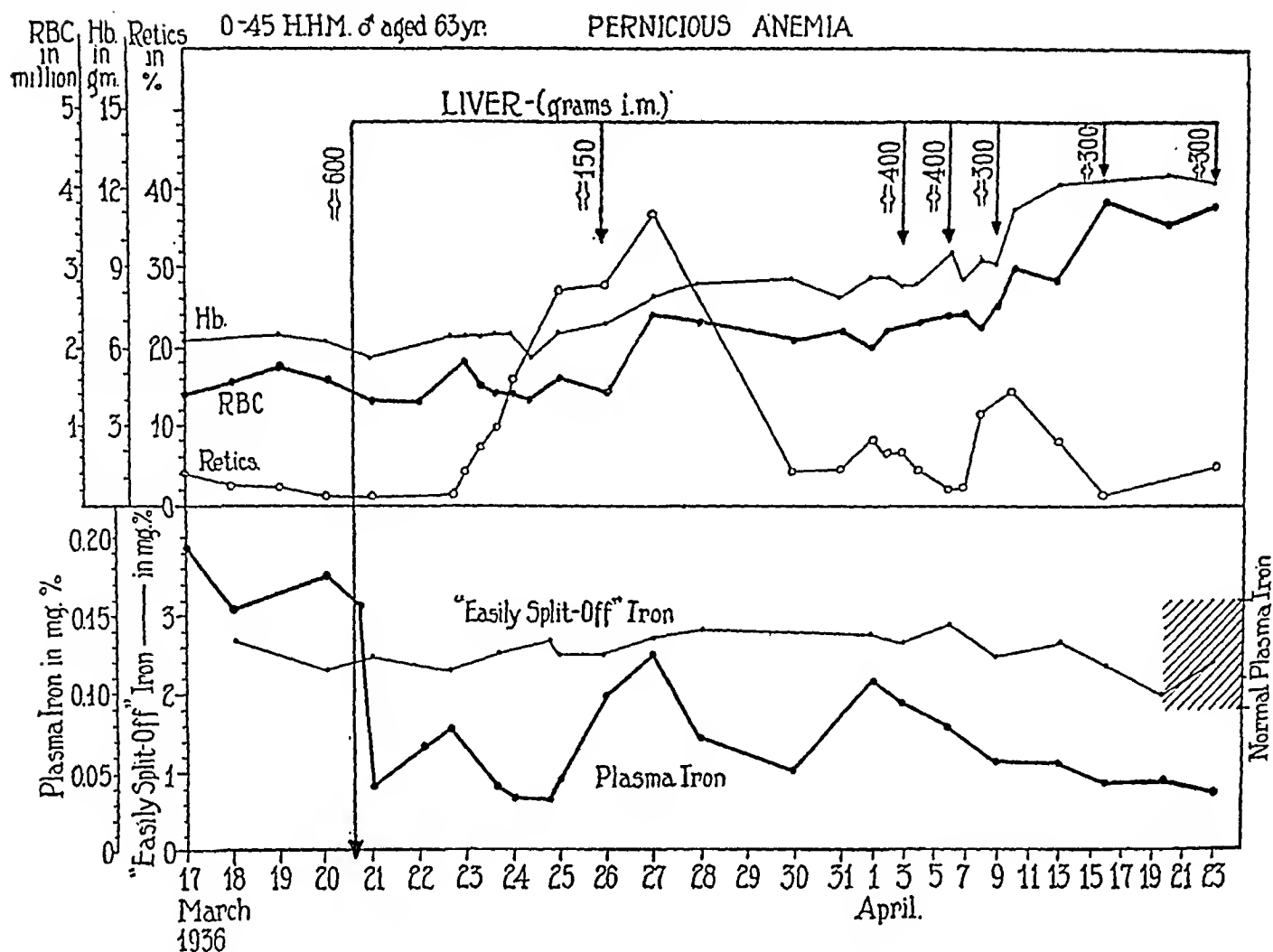


FIG. 9. EFFECT OF LIVER INDUCED REMISSION ON SERUM IRON AND "EASILY SPLIT-OFF" IRON IN PERNICIOUS ANEMIA

Patient was relatively refractory to the usual dosage of liver extract.

iron increased gradually from approximately 1.4 to 2.0 mgm. per cent (determined with 0.1 N HCl). It is interesting that the concentration of serum iron of the umbilical cord blood at the time of delivery was 247 micrograms per cent, while the serum iron in a sample of the patient's blood taken at approximately the same time was only 58 micrograms per cent. Following delivery, the red cells of the mother continued on their upward trend ultimately reaching the five million level. The hemoglobin, however, tended to lag behind more and more until a definite hypochromia of the cells developed. Iron and ammonium citrate was then given for a period of 4 weeks during which time both hemoglobin and plasma iron returned to normal.

During the greater part, at least, of the last trimester of this pregnancy, the serum iron was at a high level. Therefore, if serum iron is

transport iron, as the present authors believe, the fetus was being well supplied with adequate amounts of the metal for storage in spite of the maternal anemia. Attention, likewise, is called again to the fact that the serum iron in the cord blood at birth was 247 micrograms per cent, while that in the mother's circulating blood was only 58 micrograms per cent. The infant at birth had only 4.2 million red cells and 15 grams of hemoglobin, but within 48 hours the red cells had risen (presumably as the result of dehydration) to nearly 7.0 million and the hemoglobin to above 20.0 grams. This high level was maintained for three days, and then the usual gradual fall in erythroid elements occurred. Within a month after birth, the red cells had decreased to 4.5 million and the hemoglobin to 12.5 grams. Counts obtained during the subsequent two and a half months were slightly above these "low"

# PERNICIOUS ANEMIA OF PREGNANCY.

DIETARY DEFICIENCY OF "EXTRINSIC  
FACTOR".

0-3097 G.H.♀ Aged 21 years.  
para five.

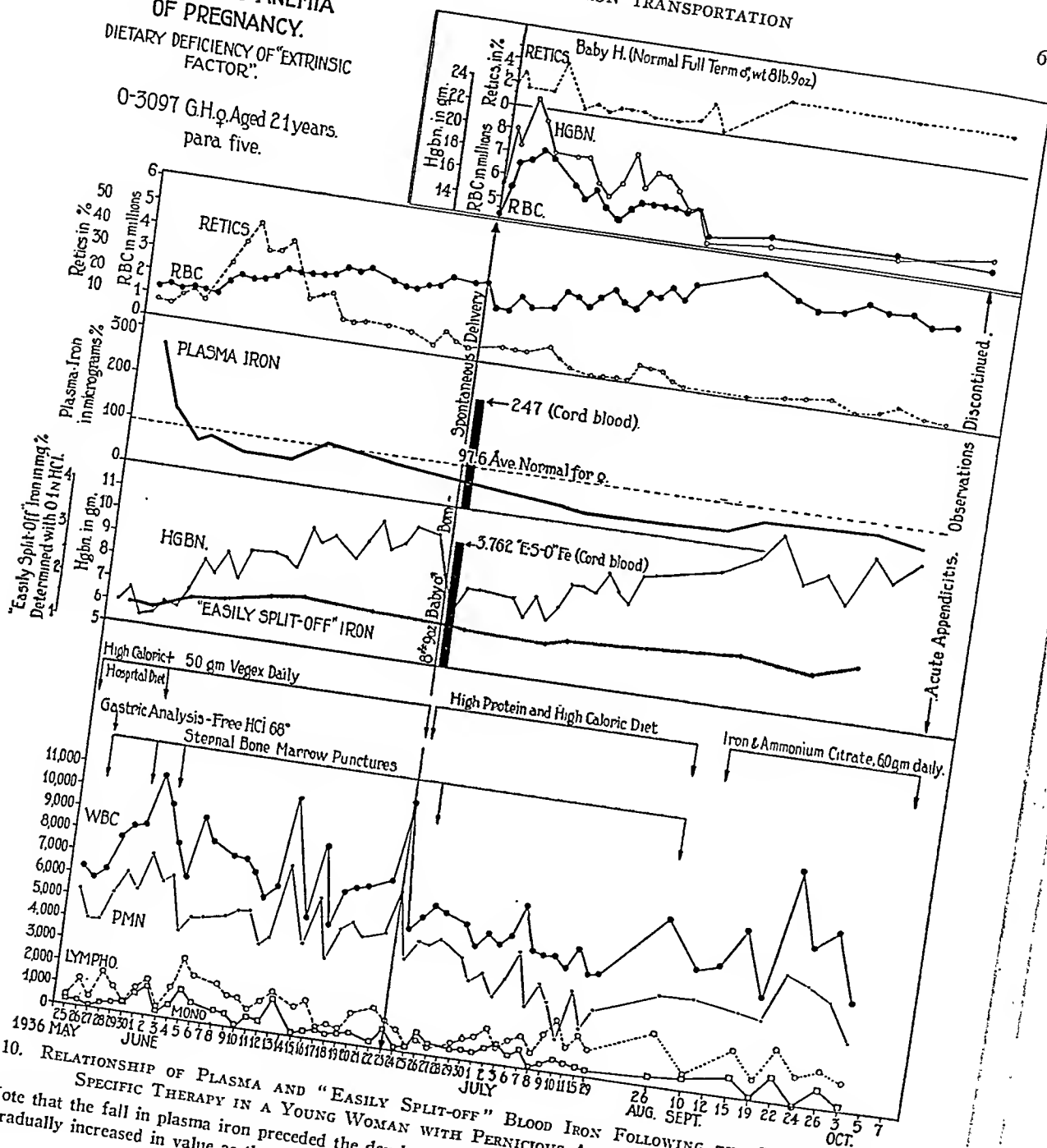


FIG. 10. RELATIONSHIP OF PLASMA AND "EASILY SPLIT-OFF" BLOOD IRON FOLLOWING THE INSTITUTION OF SPECIFIC THERAPY IN A YOUNG WOMAN WITH PERNICIOUS ANEMIA OF PREGNANCY

Note that the fall in plasma iron preceded the development of a reticulocytosis, and that the "easily split-off" iron gradually increased in value as the erythroid function returned to normal in the peripheral blood.

values. It is interesting to observe that at birth a macrocytosis with moderate variation in size and shape of the cells and a high color index were present. The fact that fall in red cells and hemo-

globin was no greater than that which occurs in infants born of "normal" mothers constitutes important clinical evidence tending to substantiate the assumption made above—that adequate

0-3097 G.H. Aged 21yr ♀ PERNICIOUS ANEMIA OF PREGNANCY

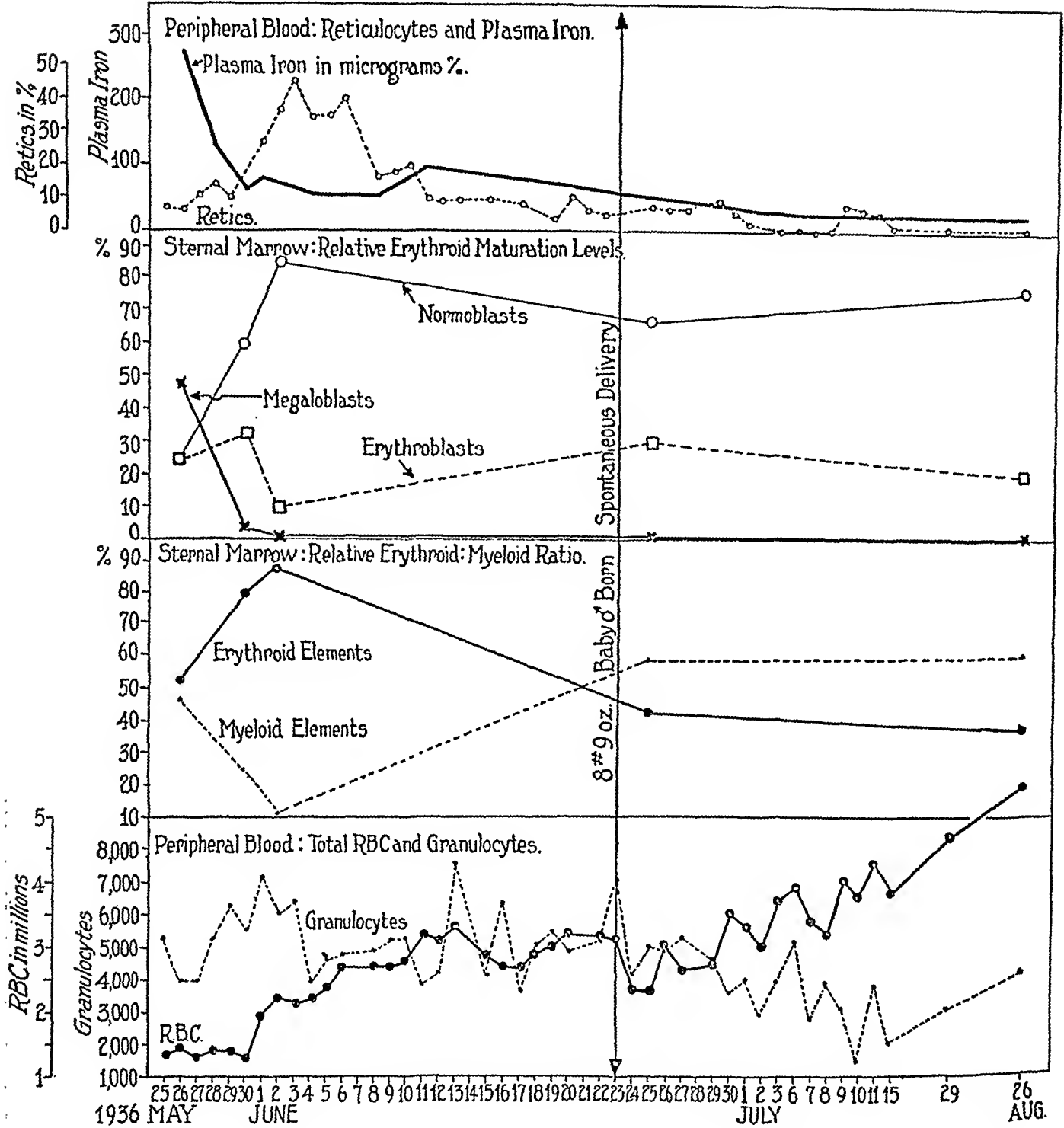


FIG. 11. FURTHER ANALYSIS OF SERUM IRON, BONE MARROW, AND CHANGES IN PERIPHERAL BLOOD CELLS IN THE CASE PRESENTED IN FIGURE 10

The fall in serum iron paralleled the disappearance of megaloblasts from the bone marrow and occurred coincident with the reinstitution of normal erythrocyte maturation and hemoglobin synthesis.

amounts of iron had been made available, through the elevated serum iron, to the fetal organism for storage.

Several less complete studies of the blood iron relationships in pernicious anemia have been made by other investigators. Erben in 1900 (24) and

Fowell in 1912 (25) reported that they had found the non-hemoglobin iron increased in pernicious anemia. Riecker (8, a) observed a high serum iron level in most of his cases and noted that after induced remissions these values returned to normal. Attention has been called (17)

however, to the fact that the serum iron values reported by Riecker are considerably higher than those obtained in more recent investigations. Dominici (16) obtained values for the "easily split-off" blood iron fraction of from 1.34 to 2.08 mgm. per cent (determined with  $\text{H}_2\text{SO}_4$ ), values with which our own figures are in close agreement.

### *The hemolytic states*

In hemolytic states, another excellent opportunity for the study of iron in the blood is provided. With an increase in red blood cell destruction, a greater amount of iron from the released hemoglobin is poured into the blood stream for transportation to organs of storage, utilization, and excretion. At the same time, the erythropoietic elements in the bone marrow are stimulated to increased activity, and extract iron from the blood for the synthesis of hemoglobin at an unusually rapid rate. The amount of transport iron present in the blood at any given time must, consequently, depend on the balance which may be struck between these two abnormally stimulated mechanisms of hemolysis and erythrocytogenesis. One would expect, therefore, that single isolated determinations of the various forms of blood iron in patients presenting hemolytic phenomena would yield little valuable information—and such has proved to be the case. In patients with congenital hemolytic icterus, for instance, both serum and "easily split-off" iron values were found to lie within their respective normal zonal ranges (Table I). Occasionally, the concentration of serum iron was a little higher than normal. Dominici (16) has reported "easily split-off" iron levels in three cases of hemolytic icterus. One of these was higher than was found in any of his control subjects; the other two were well within the limits for normal.

The detailed study of individual cases, on the other hand, has yielded most interesting confirmatory data. Following splenectomy in patients with congenital hemolytic icterus, an immediate fall in the serum iron level was noted (Table I). In the instance of B. B., a young woman 26 years old, with congenital hemolytic jaundice (Figure 12), the serum iron immediately before operation was 160 micrograms per

cent. Twenty minutes after splenectomy had been performed, the serum iron concentration had fallen to 80 micrograms per cent. This fall in serum iron level continued throughout the first two postoperative days until a low level of 30 micrograms per cent was reached. As the reticulocytes fell gradually from their preoperative high level, however, the serum iron again equilibrated, returning to within the normal limits. Following splenectomy, the abnormal destruction of erythrocytes abruptly ceased, and with this source of iron to the serum or plasma stopped, the serum iron decreased to the level at which it would have been preoperatively, because of the increased bone marrow activity, had not the spleen been pouring into the blood stream large amounts of iron from the released hemoglobin. As the bone marrow once more became capable of supplying an adequate number of erythrocytes, and normal cellular equilibration was resumed, the serum iron returned to a normal level.

Another series of observations were made on E. S., a 46 year old white male with polycythemia vera, in whom intravascular hemolysis was produced by phenylhydrazine hydrochloride (Figure 13). This patient, prior to the studies described here, had been treated by therapeutic phlebotomy. The red cells, consequently, were hypochromic, and the concentration of serum iron was low (25 to 40 micrograms per cent). During the period of most rapid hemolysis, characterized by a rapid fall in erythroid elements and by an increasing reticulocytosis, the serum iron increased to twice its basal level. "Easily split-off" iron decreased slightly with the drop in red cells, but remained constantly within the limits for normal. When the rate of hemolysis diminished and the red cells began again their upward climb, serum iron returned to its pre-therapy level.

### *The possibility of an exchange of iron between "Easily split-off" and plasma blood iron fractions*

The strongest experimental evidence to support Barkan's hypothesis that "easily split-off" blood iron is transport iron and that the iron in plasma merely serves as the direct medium of exchange between it and the tissues comes from his reported observations to the effect that with the incubation of whole blood at  $37.5^\circ\text{C}$ . an increase





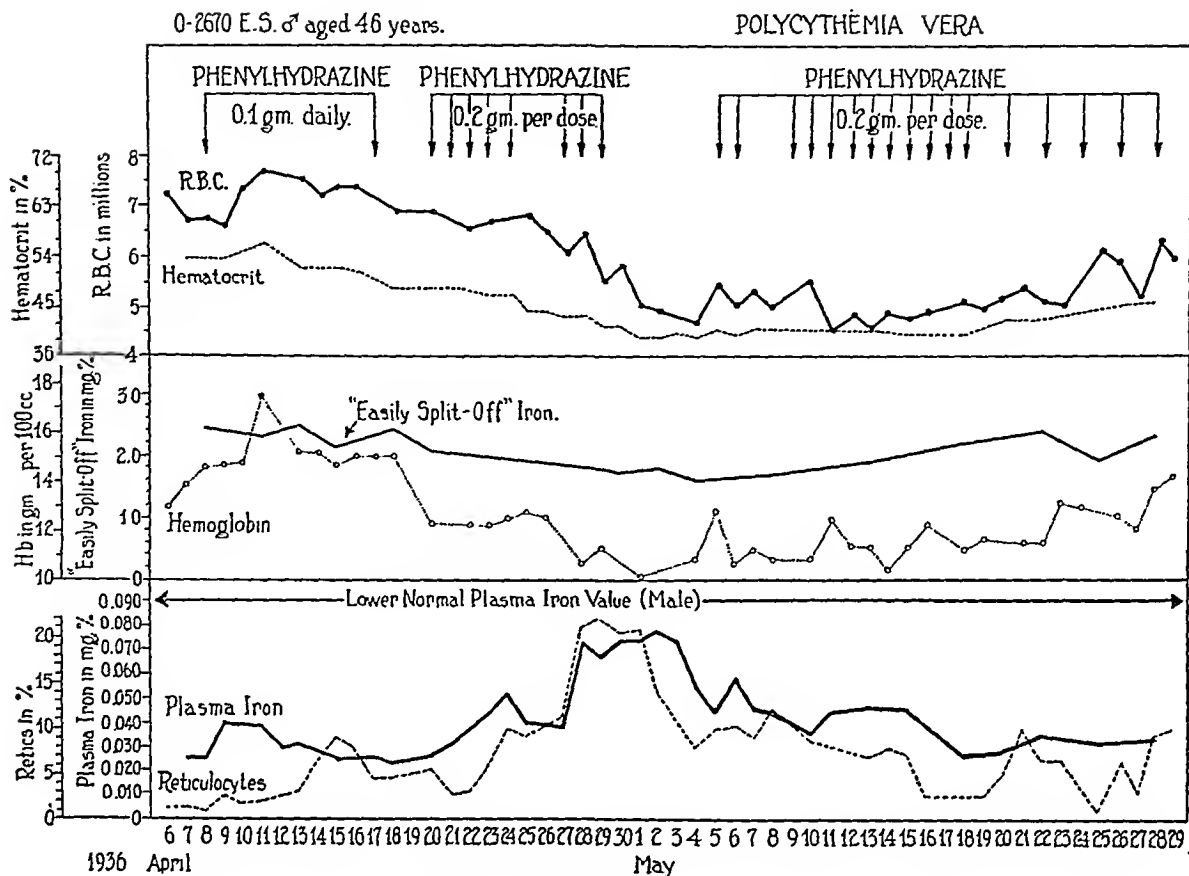


FIG. 13. THE EFFECT OF PHENYLHYDRAZINE HYDROCHLORIDE ON THE CIRCULATING ERYTHRON AND BLOOD IRON RELATIONSHIPS

Note that the period of maximum hemolysis was characterized by a rise in the reticulocytes and in the plasma iron.

in plasma iron occurs (15, b). Barkan employed the intravenous injection of sodium polyanethol-sulphonate<sup>4</sup> to make the blood of his experimental animals non-coagulable. He then collected two samples of blood. The plasma from one of these was immediately separated by centrifugation and analyzed for non-hemoglobin iron. The other specimen was incubated for six hours at body temperature and then treated in the same way. In the majority of instances, a substantial

increase (e.g. 150 to 250 micrograms per cent) in plasma iron was observed.

We have repeated these observations on both dogs and human subjects (Table II). With the former, "Liquoid" (La Roche) was used, just as in Barkan's experiments. Human blood was prevented from coagulating by being collected in flasks containing appropriate amounts of isotonic, iron-free potassium oxalate. Determinations of plasma iron were made both by digesting whole plasma in which the hemoglobin iron concentration had been estimated by the micro-benzidine method (17), and by dialysis of the plasma through cellophane membranes and subsequent digestion of the dialysate. In no case have we been able to detect an increase in non-hemoglobin plasma iron after incubation of whole blood at 37.5° C. for 6 hours. If a transfer of iron from

<sup>4</sup> Sodium polyanetholsulphonate is an organic anti-coagulant supplied by the Hoffman-La Roche Company under the trade name "Liquoid." It is used to inhibit blood coagulation in experimental animals, is active in vitro and, when administered intravenously, in vivo as well. The toxicity of the drug is demonstrated by the fact that doses of 0.02 gram or more per kilogram of body weight are fatal to the rabbit.

TABLE II

Failure of iron to be transferred from erythrocytes to plasma when whole blood was incubated "in vitro" at 37.5° C.

Subject	Anticoagulant	Plasma iron determined by digestion of whole plasma						Plasma iron determined after dialysis (acidified with 0.1N HCl)	
		Before incubation			After incubation at 37.5° C. for 6 hours			Before incubation	After incubation at 37.5° C. for 6 hours
		Total iron	Hemoglobin iron	Non-hemoglobin iron	Total iron	Hemoglobin iron	Non-hemoglobin iron		
		mgm. per cent	mgm. per cent.	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent
Dog.....	"Liquoid" (i.v.)							0.231	0.244
Dog.....	"Liquoid" (i.v.)	0.201	0.010	0.191	0.200	0.005	0.195	0.190	0.234
Dog.....	Isotonic oxalate							0.120	0.124
W. R. A. "Normal" ♂	Isotonic oxalate	0.088	0.002	0.086	0.090	0.005	0.085		
C. V. M. "Normal" ♂	Isotonic oxalate	0.099	0.002	0.097	0.125	0.020	0.105	0.102	0.104
									(0.118 after incubation at 37.5° C. for 24 hours. Gross hemolysis present.)
F. E. ♀ Polycythemia vera	None	0.036	0.001	0.035	0.039	0.002	0.037		
	Isotonic oxalate	0.031	<0.001	0.031	0.036	0.002	0.034		
E. S. Polycythemia vera ♂	Isotonic oxalate	0.042	0.006	0.036	0.093	0.049	0.044		
A. K. Polycythemia vera ♂	Isotonic oxalate	0.037	<0.001	0.037	0.043	0.004	0.039		

the "easily split-off" iron fraction to the plasma occurs, one would certainly expect it to be particularly manifest in cases of polycythemia vera in whom relative iron deficiencies had been produced by therapeutic bleeding. Under these circumstances, plasma iron was lower than normal, while "easily split-off" blood iron was either well within normal limits or at a high level. Our observations in normal animals and in normal human subjects have been supplemented with determinations in three such cases of polycythemia vera, and again no increase in non-hemoglobin plasma iron was observed.

It is not possible to argue that an exchange between the "easily split-off" and plasma blood iron fractions does not occur *in vivo*; but since such an exchange cannot consistently be demonstrated by *in vitro* observations, the possibility seems less probable.

#### SUMMARY

A brief summary of the changes effected in the plasma and "easily split-off" forms of blood iron by various endogenous and exogenous stimuli described in the experimental portion of this paper is given in Table III. Plasma or serum iron was found to be very labile. In the iron deficiency

states, it was uniformly low. Under conditions of decreased red cell formation (e.g. hypoplastic anemia and pernicious anemia in relapse), it tended to be high. When the bone marrow was stimulated to unusually active erythrocytogenesis (e.g. following acute hemorrhage and liver induced remission in pernicious anemia), the plasma iron concentration was low. In the hemolytic states, the plasma iron was found to be in equilibrium between the amounts of iron being added to the blood stream as a result of the hemolytic process, and the amount being withdrawn by the hyperplastic bone marrow. When the equilibrium was disturbed by splenectomy in congenital hemolytic icterus, the plasma iron fell to a level consistent with the rate of rapid hemoglobin synthesis. During the most active period of a phenylhydrazine induced hemolysis, the plasma iron increased in value. Following the administration of single large doses of iron salts by mouth, the concentration of iron in plasma was increased frequently to from three to ten times its basal level. Plasma iron was affected by these various influences, in other words, exactly as one would expect transport blood iron to be affected. A single graphic representation of these interrelationships is presented in Figure 14.

TABLE III

*Summary of plasma iron and "easily split-off" iron changes which relate to the question of transport iron identity*

Description	Serum or plasma iron	"Easily split-off" iron
I. Iron deficiency states . . . . .	Low	Lower than normal and normal values
II. Clinical states characterized by diminished Hb. formation (e.g. aplastic anemia and pernicious anemia in relapse) . . . . .	High	Lower than normal and normal values
III. Clinical states characterized by very rapid Hb. formation (e.g. liver induced remissions in pernicious anemia, following acute hemorrhage) . . . . .	Low	Normal (high as well as low normal values)
IV. Hemolytic anemias (e.g. congenital hemolytic icterus) . . . . .	Normal to high	Normal
V. Following large oral doses of "available" iron salts . . . . .	Increased 3 to 10 times over basal level	No change from basal level (except slight increase reflected from plasma iron increase)

"Easily split-off" blood iron, on the other hand, proved to be relatively stable. In the iron deficiency states, it was either normal or lower than normal. Under conditions of depressed hemoglobin synthesis and diminished erythrocytogenesis, it was just as likely to be low-normal as it was to be high-normal. No significant changes occurred in the fraction in patients with pernicious anemia after liver therapy. "Easily split-off" blood iron was not obviously influenced by increased rates of red cell destruction, and it did not show true increases following the administration of single oral doses of iron salts. Iron therapy in patients with iron deficiency states and slightly low "easily split-off" iron values was attended by an increase in the "easily split-off" fraction to normal levels; but this increase was relatively independent of the changes in plasma iron and erythroid elements. Low values for "easily split-off" iron occurred primarily in those anemic states in which the circulating erythroid elements were markedly decreased (particularly with hemoglobin levels of less than 5.0 or 6.0 grams). However, it is obvious from the studies following acute hemorrhage and from those fol-

lowing remissions induced by liver in pernicious anemia that no direct relationship holds between concentrations of hemoglobin and "easily split-off" iron. Investigations aimed at defining more sharply the relationship, if any, between hemoglobin and "easily split-off" blood iron are in progress.

An exchange of iron from the "easily split-off" to the plasma iron fraction was not observed after incubation of unclothed blood at 37.5° C. for 6 to 8 hour periods.

### CONCLUSIONS

1. Iron is transported as plasma iron. The quantity present in peripheral blood is influenced by, and is a measure of: (a) The amount of iron being absorbed from the gastro-intestinal tract; (b) The extent and adequacy of the iron reserves of the body; (c) The ability of the bone marrow to utilize iron in the synthesis of hemoglobin; (d) The rate of hemoglobin synthesis; (e) The extent of hemolysis taking place in spleen and other tissues; and (f) The physiological or pathological equilibrium existing between (d) and (e).

2. The physiological function of "easily split-off" blood iron remains yet to be defined.

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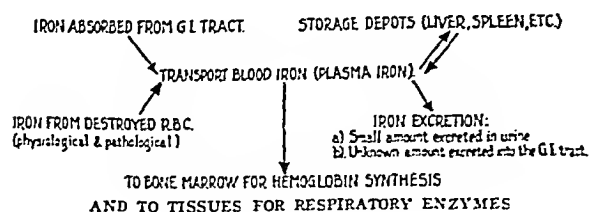


FIG. 14

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# THE RESULT OF INTRA-ARTERIAL INJECTION OF VASODILATING DRUGS ON THE CIRCULATION: OBSERVATIONS ON VASOMOTOR GRADIENT

By EDGAR V. ALLEN AND GEORGE R. CRISLER

(From the Division of Medicine, The Mayo Clinic, Rochester, Minn.)

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The effect of vasodilating drugs is ordinarily short; such drugs frequently cannot be given orally, intravenously, or intramuscularly in amounts sufficient to effect marked vasodilatation because the general systemic effects are so great that administration of such amounts is inadvisable or prohibited. It was because of this consideration that the effects of injection of drugs directly into the arteries supplying the upper and lower extremities were studied. It is apparent that the concentration of a drug within the artery and its arterioles is much greater when the drug is injected directly into an artery than it is when the drug is injected into a vein or muscle, or taken by mouth. By the intra-arterial method of administration we hoped the drugs would be "fixed" in the extremity; this would allow the use of amounts which would produce marked vasodilatation in a single extremity without causing general systemic effects. Incidental observations lead us to a consideration of the difference of the ease with which vasodilatation can be induced in the upper and lower extremities.

## METHOD OF OBSERVATION

In each instance the patient lay quietly in a room, the temperature and humidity of which were relatively constant for a few minutes before the observations were begun. Subsequent to injection the patient lay quietly until the observation was completed. In all instances the temperature of the skin was determined by the Sheard electric thermometer; a thermocouple was placed on a digit of each extremity. The skin overlying and the tissues surrounding the brachial and femoral arteries were infiltrated with a few cubic centimeters of a 2 per cent solution of procaine. The arteries were punctured with an ordinary venipuncture needle attached to a syringe containing 1 to 5 cc. of the solution of the drug to be injected. Papaverine hydrochloride in doses of 0.032 to 0.065 gram histamine phosphate in doses

of 0.1 to 0.15 mgm., and Acetyl B-methylcholine (mecholyl) in doses of 0.5 to 2.0 mgm. were injected intra-arterially in the various cases. Only cases in which the temperatures of the digits were several degrees centigrade less than that attainable by vasodilatation were used in the final compilation, since it is apparent that when the temperature of the skin of a digit is high, which indicates marked vasodilatation, additional vasodilatation can be only minimal or cannot be induced.

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A solution of papaverine hydrochloride was injected into the brachial artery in ten cases. There was no detectable impairment of circulation to the upper extremities in any of these cases. There were four cases in which chronic occlusive arterial disease affected the lower extremities, and in the determination of averages shown in Figure 1, observation on the temperature of the toes in these cases was excluded. The remaining individuals were well or had hypertension or chronic arthritis. The usual local response was an immediate blush which involved the entire extremity distal from a level slightly above the site of injection. In a few minutes the temperature of the fingers supplied by the artery which was the site of the injection began to increase and a few minutes later the temperature of the digits of the opposite extremity began to increase. The temperature of the toes seldom increased and in several cases it actually decreased. While considerable variation in response of digital temperatures was noted in the various instances, the trend of change was almost uniform. The results of the ten observations are presented in the composite graph at the left of Figure 1. These observations seemed to indicate that the papaverine was "fixed" in the upper extremities, which were the site of the injection, since the average temperatures of the toes



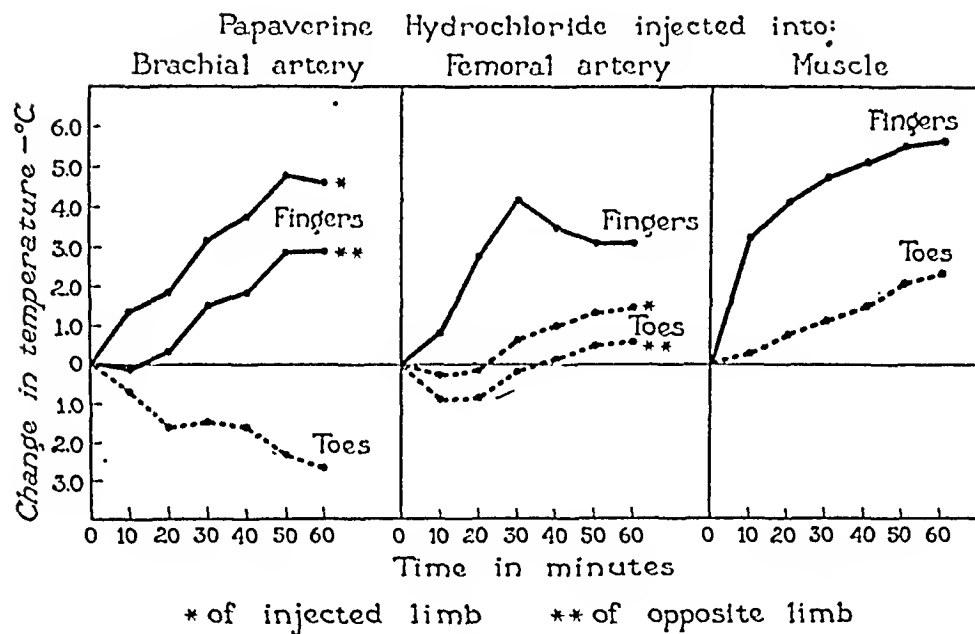


FIG. 1

The temperature of the toes decreased after injection of papaverine hydrochloride into brachial arteries while that of the fingers increased. However, injection into femoral arteries caused a smaller increase of temperature of the toes than of the fingers and produced effects similar in this regard to intramuscular injection of papaverine. The vasodilating effect of papaverine on the toes seems to be enhanced very little if at all on injecting it into the femoral artery.

did not increase. Subsequently, the drug was injected into the femoral artery in eight cases. Six of these were cases of chronic arthritis without hypertension or impairment of the circulation to the lower extremities, and two were cases of chronic occlusive arterial disease. Since the response of the temperature of the toes in these two instances was roughly the same as that of the toes in cases in which occlusive arterial disease was not present, the results are included in this study. The blush noted when the drug was injected into the brachial artery was usually entirely absent but occasionally occurred very faintly. Surprisingly, the average increase in temperature of the lower extremities was slight, while that in the fingers was much greater. While the response noted varied considerably in individual cases, the trend of change in temperature was roughly the same in each case. The results of the eight observations are shown in a composite graph in the central part of Figure 1. The results given for the fingers are in each instance the average of the increases in temperature of one finger on each hand.

#### *The intramuscular injection of papaverine hydrochloride*

Papaverine hydrochloride in doses varying from 0.097 to 0.2 gram was injected intramuscu-

larly in ten cases. Eight of the patients in these cases had Raynaud's disease, chronic arthritis, jaundice, or hypertension, without occlusive arterial disease, and two had occlusive arterial disease of the lower extremities. Since the changes in the temperature of the skin of the toes of patients who had occlusive arterial disease followed roughly the changes noted in the other observations, they are included in this report. The average response of the temperature of fingers and toes charted at the right of Figure 1 indicates a rapid and marked increase in the temperature of the fingers and a slower and less marked increase of the temperature of the skin of the feet. Subsequently, 0.097 gram of papaverine hydrochloride was injected intravenously. The temperature of the skin of the fingers increased 7.2° C. in fifty minutes while that of the toes decreased almost 1.0° C.

#### *The intra-arterial injection of Acetyl B-methylcholine (mecholy)*

Acetyl B-methylcholine (mecholy) was injected into the brachial arteries of four patients, three of whom had no occlusive arterial disease and one of whom had occlusive arterial disease in one leg, the digits of which were not used for the observation of the temperature. As in the ob-

servations on the effect of papaverine, immediately following the injection of mecholyl, an intense blush involved the entire arm distally from a level above the point of injection. The average temperature of the digits of the hand on the side of the injection increased in a few minutes, and the temperature of the digits continued to increase. The temperature of the fingers on the opposite hand likewise increased, but did so more irregularly and more slowly. The temperature of the toes increased to a much smaller degree. The results are shown in a composite graph in the left part of Figure 2. Mecholyl was then injected

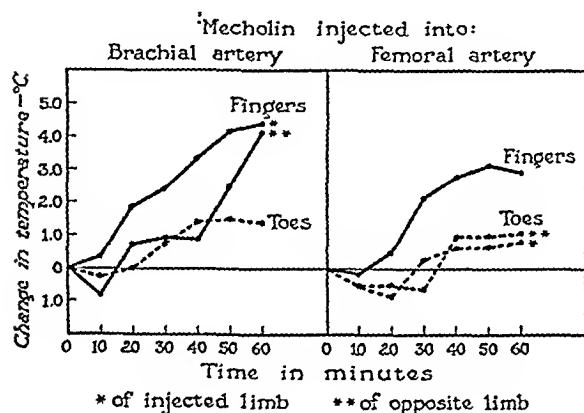


Fig. 2

Regardless of whether mecholyl is injected into brachial arteries or femoral arteries, vasodilatation is more marked in the fingers. The vasodilating property of mecholyl on any extremity is largely independent of whether it is injected into the artery supplying that extremity or into another artery.

into the femoral arteries of three patients, two of whom had arthritis. The results are shown in a composite graph at the right of Figure 2. As in the case of the intrabrachial injection of papaverine, the temperature of the skin of the fingers increased very much more than did that of the toes.

#### The intra-arterial injection of histamine

Histamine was injected into the brachial arteries of five patients. One of these patients had arthritis, one had multiple sclerosis, one had polycythemia, and two had thrombo-angiitis obliterans of the lower extremities. Only the temperature of the skin of the toes of the three cases in which occlusive vascular disease was absent was ac-

cepted for final calculations. As in the observations with papaverine and mecholyl, an intense blush involved the skin supplied by the artery into which histamine had been injected, and the temperature of the extremity increased rapidly. The temperature of the digits of the opposite extremity increased slowly and less markedly, while that of the toes was influenced very little. The temperature of the fingers on the side of the injection increased an average of 5.0° C.; the temperature of the fingers of the opposite side increased 3.0° C., while the temperature of the toes decreased 0.4° C. in thirty minutes, but in the following twenty minutes it increased to 1.6° C. more than the temperature just before the injection.

#### SUMMARY

The influence on temperature of the skin of digits of all extremities following the injection of histamine, papaverine, and mecholyl into the brachial artery and into the femoral artery are shown in Figure 3. It is apparent from these

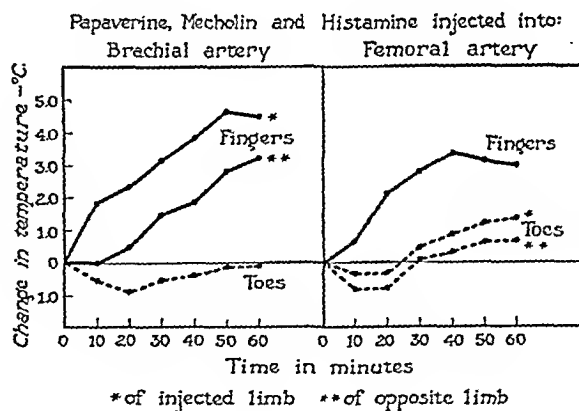


Fig. 3

The toes are relatively refractory to the vasodilating action of papaverine hydrochloride, mecholyl, and histamine, for increases in temperature of fingers are in composite significantly greater than those of toes, regardless of whether these drugs are injected into the brachial artery or the femoral artery.

observations that it is impossible to "fix" a vasodilating drug in an extremity to any great extent by injecting the drug into the artery supplying the digits. The effects on the temperature of the skin of the digits supplied by the artery into which the drug was injected and the effects on the

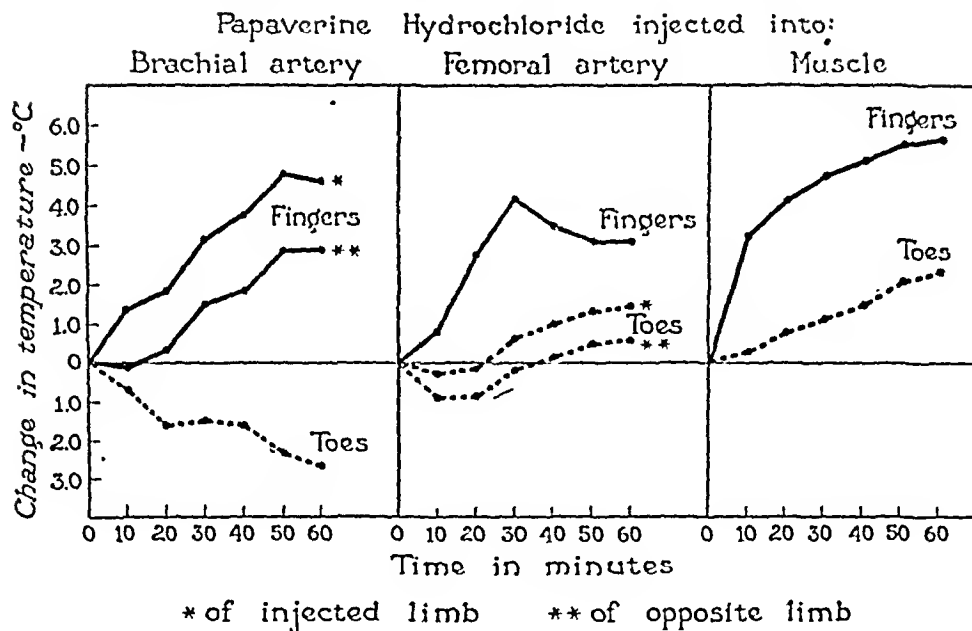


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collection during the test would result in large errors. Such errors have undoubtedly crept into some of the cases reported here. However, this error cannot explain the consistent findings shown in Figure 1. The points, with few exceptions, are fairly uniform in distribution and with decreasing urine volume progressively fall lower and lower on the graph. Every clearance observed with low urine volumes, during 1935 and 1936, is shown in Figure 1, which therefore represents wholly unselected cases.

In 12 patients and 3 normals, three or more successive hourly clearances were done. These are instructive. In some cases, two closely checking clearances were obtained with urine volumes above the critical limit, while the clearance obtained with the small urine volume was much lower. In these cases, one can hardly attribute the low clearance to incomplete collection of the urine. In other cases, with two different volumes below 0.35 ml., and one above, the clearance rises with the increase in volume. In still other instances, low clearances check as calculated from similar low volumes, while a high clearance is observed above the critical volume.

Usually the higher urine volume and higher clearance were observed in the second of two or the last of three hourly clearances. This is because the patient was given extra water if the first urine volume was low. A still greater factor is that she was beginning belatedly to excrete the previously ingested water. This slow excretion of water is a well known characteristic in pregnancy toxemia. But in 36 cases, the first urine specimen had the higher volume and showed the higher clearance.

Apparently the critical urine volume is about 0.35 ml. per minute, or possibly a little higher (21 ml. per hour or more). This checks fairly well with Maddock and Coller's (6) recalculation of Lashmet and Newburgh's observations. They show that with an upper specific gravity of 1.024, there must be voided at least 605 ml. of urine in twenty-four hours if 35 grams of waste are to be excreted. When a specific gravity of 1.032 is attained, at least 473 ml. are needed. (These volumes would be 0.42 and 0.329 ml. per minute.)

The critical urine volume, below which the square root rule fails, apparently varies with the individual. Van Slyke has found a similar varia-

TABLE 1

*The effect of low urine volume upon the calculated standard urea clearance. Determinations made in successive hours. Normal adults and patients with pregnancy toxemia. Also shown in Figure 1.*

Vol- ume output	Calcu- lated C <sub>s</sub>	U/B	Blood urea nitrogen	Vol- ume output	Calcu- lated C <sub>s</sub>	U/B	Blood urea ni- trogen
ml. per minute	per cent		mgm. per 100 cc.	ml. per minute	per cent		mgm. per 100 cc.
0.262	68	72	(Normal) 22.0	0.333	125	117	7.5
0.323	74	71		0.164	68	92	
0.258	54	57		0.733	151	97	
0.313	66	63		0.750	142	89	
0.517	101	74		0.216	77	90	
0.173	79	103	(Normal) 12.4	0.458	102	82	8.6
0.234	89	99		0.184	62	79	
0.250	100	108		0.267	89	93	
0.225	94	107		0.524	121	91	
0.167	67	88		0.167	58	77	
1.100	110	57	(Normal) 12.4	0.517	89	67	9.5
13.950	109			0.968	84	46	
0.173	82	106		0.083	14	28	
0.458	128	103		0.191	48	59	
0.150	45	63		1.000	158	86	
0.450	102	82	13.2	0.233	49	56	7.9
0.500	92	69		0.567	76	54	
0.115	24	79		0.113	28	42	
6.700	71			0.700	72	46	
9.250	67			0.162	14	18	
0.182	34	43	11.6	0.429	28	24	26.7
0.241	42	46					
1.350	81						

tion in the augmentation limit. This difference may be seen in the normals A and B, Table I. By virtue of a higher urea concentration ratio, B was able to maintain a urea clearance of 100 per cent with a minute urine volume of 0.25 ml. A, with the same urine volume, showed a clearance of 54 per cent.

The reason for the failure of the square root law at these low urine volumes is not merely that the urea concentration fails to increase with the decreasing volume, as postulated. As the urine volume continues to fall, the urea concentration ratio reverses its trend and now decreases. This is shown in many of the cases in Table I.

#### DISCUSSION

Calculation of the standard clearance from very small urine volumes apparently will give results considerably too low. This has been a stumbling block in several papers, particularly in those discussing renal function in pregnancy toxemia.

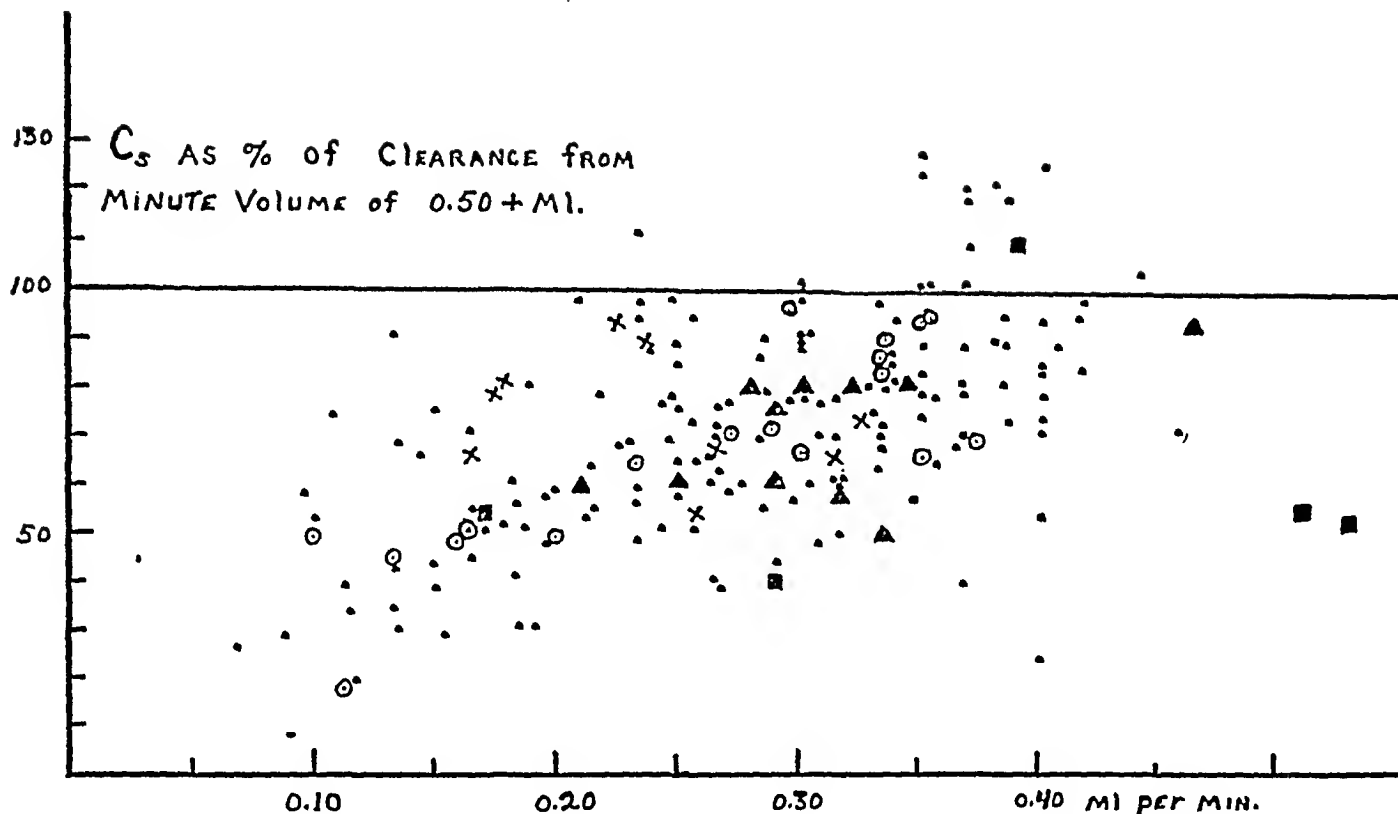


FIG. 1. THE INFLUENCE OF URINE VOLUME UPON THE CALCULATED UREA CLEARANCE

The standard clearance calculated from the lower or lowest urine volume is plotted as per cent of the clearance calculated from a volume of 0.50 ml. or more.

- Pregnancy toxemia.
- ⊙ Bright's disease complicated by pregnancy.
- × Normal.
- ▲ Data of Möller, McIntosh, and Van Slyke (1, 3) and Austin, Stillman, and Van Slyke (2) for normal and nephritic individuals.
- Data of Cullen, Nelson, and Holmes (5) for nephritic children.

#### RESULTS

The observations in 169 cases of pregnancy toxemia are summarized in Figure 1. This figure also shows all of the relevant data published in the papers alluded to above, together with a recalculation of Cullen, Nelson, and Holmes' (5) observations on nephritic children. In the graph, the patient's lower or lowest clearance has been plotted as per cent of the higher or highest clearance determined in the preceding or succeeding hour(s). This was done so as to include data from chronic nephritics.

It is obvious from the graph that the lower the minute urine volume, below about 0.35 ml., the greater the downward deviation of the calculated standard clearance below the level computed from the higher urine volume. This limiting volume, of about 0.35 ml., we shall call the *critical volume*. Below it the square root law does not appear to

be valid, and the farther the volume sinks below it the greater becomes the error in calculating the standard clearance.

The distribution of the different symbols on the graph indicates that the toxemia patients are not peculiar in regard to this deviation from the square root rule of urea excretion. Chronic nephritic patients show the same dependence upon urine volume for the maintenance of a urea clearance level predicted from the rule. The four normal adults studied show the same dependence. Furthermore, these data find support in the published work of Van Slyke and his collaborators. Most of Cullen's observations fall below the mean range of the other points; perhaps this is because the corrected urine volume rather than the actual volume was plotted.

Since we are dealing with small urine volumes, the failure completely to drain the bladder at any

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*The Etiological Relationship of the Eosinophile to the Gordon Test for Hodgkin's Disease.* By JOSEPH C. TURNER (by invitation) and HENRY JACKSON, JR., Boston, Mass.

In 1932 Gordon reported that broth suspensions of lymphadenomatous (Hodgkin's) tissues upon inoculation into the brains of rabbits or guinea pigs produce a characteristic paralysis (Gordon test). Subsequent investigation has established that approximately 75 per cent of cases of Hodgkin's disease exhibit this phenomenon. Friedemann (1933) discovered that a paralysis in the same animals, indistinguishable from that of Gordon, is produced with extracts of normal human bone marrow, spleen, or leukocytes.

The nature and significance of the paralysis-producing agents of Gordon and of Friedemann remain obscure. Their identity has not been proved.

We have performed the Gordon test in ten cases of Hodgkin's disease. The test was positive in six and in these six only could eosinophiles be found. Moreover, the potency of the material used varied directly with the number of eosinophiles present.

In order to test the significance of this observation, suspensions were made of the bloods of normal and diseased individuals, some of whom had a high degree of eosinophilia. Animal inoculations showed that a paralysis-producing factor was present in titers which paralleled precisely the number of eosinophiles in suspension. Suspensions containing few, or no, eosinophiles gave negative results.

It appears that the Gordon test is positive in Hodgkin's disease only because of the presence of eosinophiles. It is tentatively proposed that the paralysis-producing agent present in the tissues of certain cases of Hodgkin's disease and that present in normal leukocytes are both derived specifically from the eosinophile and are therefore identical.

*Studies of a Stable Antigen of Vaccine Virus.* By ROBERT F. PARKER (introduced by J. T. Wearn), Cleveland, Ohio.

The isolation of a serologically active heat stable substance from tissues infected with vaccine virus has already been reported, and the chemical characteristics of the substance have been described. On inoculation into rabbits, it elicits the formation of antibodies, demonstrated by precipitation and complement fixation tests. The serum agglutinates heated and unheated elementary bodies of vaccinia but apparently neutralizes only very small amounts of vaccine virus. Rabbits subjected to repeated inoculation with the substance and having a high

titer of serum antibodies, are not resistant to infection with vaccinia.

*Estimation of Thyrotropic Hormone in Human Urine and Blood in Health and Disease by the Micrometric Analysis of the Response of the Guinea Pig Thyroid.* By PAUL STARR and (by invitation) RULON W. RAWSON, Chicago, Ill.

A method of approximating the amount of thyrotropic hormone present in a solution by the micrometric analysis of the response of the guinea pig thyroid has been described. A frequency curve of the heights of the acinar cells measured with an ocular micrometer was plotted and subjected to statistical analysis. Increasing doses of a known thyrotropic preparation (Parke, Davis and Company) gave a series of curves shifting increasingly to the right.

The urine itself, or the acetone precipitate from small amounts, or the isoelectric precipitate from large amounts, injected into the animal, produces characteristic shift of such a curve. In the case of a normal person with a normal metabolic rate and no evidence of thyroid inactivity, there is slight shift to the right. Patients with moderately low metabolic rates, "relative hypothyroidism," have more of this material in the urine. This suggests that the procedure may be used to detect the need for thyroid hormone when general metabolic measurements are inconclusive. Following total thyroidectomy the urine contains excessive amounts.

In hyperthyroidism there is less thyrotropic hormone present than in the normal urine. This is true whether or not exophthalmos is present. This raises the possibility of the use of the procedure as a diagnostic aid in hyperthyroidism. Patients with various types of pituitary tumors and goiters have different amounts of the material in the urine.

The possibility that lack of response to the hyperthyroid urine is due to excess iodine present has been covered by the addition of amounts of iodine of like order to specimens giving marked stimulation. The possibility that the response is not specifically due to thyrotropic hormone has been met by heating the specimen, since the pituitary thyrotropic hormone is heat labile. Furthermore, the hyperthyroid urine containing similar amounts of foreign material fails to give a response.

Blood serum from these groups of patients may be classified in the same way.

*Conclusion.* The method affords a convenient and specific means of assaying the appearance of thyrotropic hormone in clinical material.

For instance, Dieckmann (7) has reported urea clearances of less than 39 per cent in  $\frac{2}{3}$  of *ante partum* toxemic multiparae with no history of previous toxemia or renal disease.

This does not seem reasonable. When one sees that the blood urea nitrogen for this group was  $15.4 \pm 0.74$ , he can not accept these low clearances. The difficulty is resolved when one studies Dieckmann's Table II, Section B. The mean minute volume for the group mentioned was 0.643 ml., with a standard deviation of 0.408 ml.

Several writers have reported low urea clearances in eclampsia. Others believe that low clearances are found at the height of pregnancy toxemia. Since oliguria is very often found at the height of toxemia, one may probably attribute these findings to the oliguria and not to renal damage. The urea clearance was found by Chesley (8) to be normal in preëclampsia, eclampsia, and hypertension. The clearances did not differ significantly from values found for normal pregnant and puerperal women. No clearance was included unless calculated from a urine volume in excess of 0.35 ml. per minute.

Many of the low standard clearances calculated in acute nephritis are probably too low, because of the oliguria. This may be true in cases showing a normal specific gravity but a low clearance. According to Fishberg (9) the scanty urine of a patient with acute nephritis may show a high or a low specific gravity. If the specific gravity be high, the oliguria is of extrarenal origin, and the kidney damage is not extensive. When the specific gravity is low with oliguria, then the renal function has been measurably impaired. Alving and Van Slyke (10) found the specific gravity test to be a more sensitive measure of kidney impairment than is the urea clearance. This, they say, is also true in acute nephritis.

#### SUMMARY AND CONCLUSIONS

The square root formula for calculating the standard urea clearance gives erroneously low results when the urine flow falls below a critical volume, which for adults is about 0.35 ml. per minute. When the urine collected in an hour's

clearance test falls below 20 cc., the test should be discarded.

Below this critical urine volume, the U/B reverses its trend and decreases with further volume decrease.

The influence of small urine volume upon the calculated standard clearance is discussed with regard to findings in pregnancy toxemia and acute nephritis.

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*Influence of the Thyroid Gland on Intestinal Absorption of Dextrose, Galactose, and Xylose.* By T. L. ALTHAUSEN, with the technical assistance of M. STOCKHOLM, San Francisco, Calif.

Normal rats, rats treated with thyroxin, and thyroidectomized rats were given measured amounts of sugar by stomach tube. The amount of sugar absorbed in one hour was calculated from the unabsorbed residue in the digestive tract. In hyperthyroidism, intestinal absorption of dextrose and of galactose was markedly increased. In hypothyroidism, intestinal absorption of dextrose was reduced considerably.

Depletion of carbohydrates, raised basal metabolism, increased velocity of the blood flow, intestinal hyperperistalsis, and rate of gastric discharge appear to play no part in the observed changes of intestinal absorption. Therefore, a specific action of thyroid hormone on the intestinal mucosa is suggested as a possible explanation.

Increased phosphorylation appears to be the chief mechanism by which thyroxin increases absorption of dextrose because phlorizin which inhibits this mechanism greatly decreases the absorption of dextrose in experimental hyperthyroidism. But simple diffusion also seems to play a part since absorption of xylose is likewise increased by thyroxin.

Clinically, altered intestinal absorption may be an important factor in producing abnormally high dextrose tolerance curves often seen in patients with hyperthyroidism and unusually low curves observed in patients with myxedema. The same mechanism may play a major part in the impairment of "tolerance" to galactose observed in hyperthyroidism in man.

*Pressor Effects of Renal Extracts of Normal Dogs and of Dogs with Experimental Renal Hypertension.* By T. R. HARRISON, ALFRED BLALOCK and (by invitation) M. F. MASON and J. R. WILLIAMS, JR., Nashville, Tenn.

Hypertension has been produced in dogs by the method of Goldblatt (partial occlusion of the renal arteries). Saline extracts of the kidneys of such animals have been injected into rats and the changes in blood pressure compared with those produced by extracts of the kidneys of normal dogs. The former extracts were found to have greater pressor effects.

In a second series of experiments preparations from the two kidneys of a dog rendered hypertensive by the application of a clamp to one renal artery were studied. Here again the extract of the ischemic kidney caused a greater rise in blood pressure than did that of the normal kidney.

*Maintenance of the Functional Integrity of the Occluded Large Arteries as Demonstrated by Thorotrast Arteriography.* By WALLACE M. YATER, Washington, D. C.

Arteriography is teaching us many things about the mechanism of the circulation of vascular disease which are not demonstrable otherwise. Large arteries may become completely occluded in some part of their course

without serious trophic disturbance of a permanent nature. Arteriograms in such cases often show direct anastomosis by means of smaller arteries between the segments of the artery above and below the area of occlusion. In such cases the roentgenograms show that the artery below the obturated portion is still functioning, the blood having been shunted around the occluded portion. Arteriograms and drawings of the extremities of patients illustrating this mechanism of readjustment of the circulation are shown. Various types of anastomoses are demonstrated.

*The Effects on the Cardiovascular System of Fluids Administered Intravenously.* By MARK D. ALTSCHULE and DOROTHY R. GILLIGAN (introduced by Herrman L. Blumgart), Boston, Mass.

The effect on the cardiovascular system of the injection of fluids intravenously, such as is employed routinely in postoperative treatment, has been studied in a large group of patients with no evidence of heart disease. The fluids employed were physiological saline solution or 5 per cent glucose in distilled water, or 5 per cent glucose in physiological saline; the volumes injected varied from 500 to 1500 cc. and the rates of injection from 6 to 80 cc. per minute.

Immediately after the injection of fluids intravenously, the minute volume output of the heart and the blood volume were increased, and the velocity of blood flow was accelerated. When the more rapid rates of injection were employed or the larger volumes of fluid administered, significant and even marked increases in venous pressure were observed; a return to the control level of venous pressure was again observed within approximately twenty minutes after the end of injection in these patients with no evidence of heart disease. Slight increases in pulse rate and in systolic blood pressure and pulse pressure were observed in approximately 40 per cent of our studies. Electrocardiographic studies revealed in some instances appreciable increases in the height of the P and variable changes in the T-waves. The magnitude and duration of the above effects were influenced by the composition of the fluid injected.

These studies have demonstrated that the administration of fluids intravenously under conditions frequently employed routinely subject the heart to increased work, which amounts to about 50 per cent above basal levels. The implications of these findings in the management of patients with cardiovascular disease is clear.

*The Estimation of the Subcutaneous Tissue Pressure by a Direct Method.* By G. E. BURCH and W. A. SODEMAN (introduced by J. H. Musser), New Orleans, La.

Direct determinations of tissue pressure have not been reported in this country. Wide variations reported in the German literature have prompted us to determine directly the tissue pressure by the simple manometric method employed by Yandell Henderson for the determination of muscle tone. A 26-gauge needle was used in place of a 20. In each determination three readings were taken which agreed within  $\pm 1$  mm. of water.

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In 10 normal adults the mean values at heart level varied from 17.9 to 37.1 mm. of water in the subcutaneous tissue of the dorsum of hand and foot, volar surface of the forearm and the pretibial area. The tissue pressure in the dorsum of the foot was increased in the erect position and further increased by weight bearing. Six normal subjects stood for one hour against a table inclined at 75°. The mean tissue pressure reading in the dorsum of the foot at the beginning of the hour was 53.5 mm. of water, and at the end 65.2. In all cases there was a rise on standing for one hour not to exceed 31 mm. of water. The effect of venous pressure on tissue pressure readings was determined by inflating a blood pressure cuff around the arm in steps of 135 mm. of water (10 mm. Hg) up to 810 mm. of water (60 mm. Hg) at two minute intervals. In no case was the diastolic arterial blood pressure exceeded. Increase in venous pressure over short intervals of time had relatively slight effect upon the tissue pressure readings. In 10 patients with increasing cardiac edema the tissue pressure was greatly increased. In receding cardiac edema the values were lowered, even below normal, to return finally to a normal level.

These findings indicate: (a) Normal tissue pressure is less than the accepted values for capillary and venule pressures; (b) tissue pressure is an important factor in the control of the movement of fluid between blood vessels and tissue spaces; (c) the full effect of venous pressure on tissue pressure is not immediate; up to one hour the effect of venous pressure upon tissue pressure is slight but in congestive heart failure with prolonged high venous pressure the tissue pressure is greatly elevated; (d) the tissue pressure in patients with cardiac edema varies with the state of the edema, and (e) factors other than venous pressure are important in the regulation of tissue pressure.

*Studies of the Salt and Water Hormone of the Adrenal Cortex.* By GEORGE A. HARROP and (by invitation) GEORGE W. THORN, Baltimore, Md.

Injections of adrenal cortical hormone have been shown to produce a marked effect on the renal excretion of sodium and potassium in normal male dogs, maintained under uniform conditions with a constant fluid and mineral intake. A subcutaneous injection resulted in an immediate marked reduction in renal sodium excretion accompanied by an increased potassium excretion. Subcutaneous injections produced a much more prolonged action than the intravenous injection of a similar quantity of hormone. Subcutaneous injections twice daily effected a marked reduction in sodium excretion during the twenty-four hour period, followed by a "rebound" effect during the second twenty-four hours in which the increased excretion of sodium approximated the initial retention. It has been demonstrated that this response is proportional to the quantity of hormone given, and that the normal dog can thus be used for assay of the "salt and water hormone" effect of adrenal cortical extracts. Individual animals vary in their response to a given quan-

tity of extract, injected twice daily, but the same quantity of extract consistently produces the same change in a given animal.

A method for the extraction of the "salt and water" hormone from urine has been employed, based on extraction of urine with ethylene dichloride and subsequent distillation at a temperature of 40° C. under reduced pressure. An alcoholic extraction of the residue was distilled at the same temperature, the final residue being taken up in normal saline solution and sterilized by passage through a Berkefeld filter. Extracts of urine to which hormone had been added yielded a 40 per cent recovery when tested by the above method of assay. Acid hydrolysis of the urine, to which adrenal cortical extract had been added, almost completely destroyed the activity of the "salt and water" hormone. Intravenous injections of large quantities of adrenal cortical hormone (3000 dogs units) into normal dogs were followed by only 10 per cent recovery from the urine. Two liter portions of urine obtained from normal male, female, pregnant dog and man, and pregnant mare have been extracted and tested for the presence of the salt and water hormone. No appreciable amount could be demonstrated from this volume of urine.

Studies reported elsewhere have indicated that in the intact dog a sodium sparing effect is common to several crystalline sex hormones and closely related compounds. It is apparent that substances other than the adrenal cortical hormone exert a sodium sparing effect in the intact animal. For this reason, the method of assay which has been described is limited to the determination of the relative "salt and water hormone" content, of adrenal cortical extracts.

*Chloride Depletion in Conditions Other Than Addison's Disease.* By ALEXANDER W. WINKLER and ORRIN F. CRANKSHAW (introduced by John P. Peters), New Haven, Conn.

Extensive studies of salt metabolism in two cases not having Addison's disease showed persistent urinary Cl excretion at abnormally low Na and Cl concentration in the serum. One was a case of pulmonary tuberculosis, the other a case of primary carcinoma of the lung. In a series of other cases of tuberculosis without Addison's disease, including three cases which subsequently came to autopsy, determinations of Na and Cl in the serum were made. Consistently subnormal values were noted. The difficulties in differentiating such cases from true Addison's disease by a characteristic chemical test are noted.

*Heat Stroke: A Clinical Study.* By M. A. BLANKENHORN and E. B. FERRIS, JR., and (by invitation) G. E. CULLEN and H. W. ROBINSON, Cincinnati, Ohio.

During two successive heat waves in 1936 studies of patients suffering from heat stroke were made in the Cincinnati General Hospital and the Children's Hospital Research Foundation of the University of Cincinnati. Forty-three patients with high temperatures were received, of whom sixteen died and thirteen were autopsied.

Routine clinical studies were made on thirty-nine patients. On seventeen patients studies of the circulation, including venous pressures, were augmented by analysis of carbon dioxide content and oxygen content of arterial and venous blood to demonstrate the state of circulation. Further chemical studies of the blood were carried out, including determinations of protein concentration, hemoglobin concentration, acid base balance and cell volume.

The results of these studies demonstrated adequate circulation, a moderate but definite hemoconcentration, and a moderate acidosis. There was no striking change in the sodium chloride content of the serum in the seventeen patients who were adequately studied, which indicates that these cramps are distinctly different from the condition of "heat cramps." Absence of sweating was a constant finding on admission and most patients noted a diminution or absence of sweat preceding the onset of heat stroke. No cause for the cessation of sweating is suggested by any of these studies. It is, however, apparent that peripheral or congestive failure, deficient chlorides, and dehydration were not primary factors in the retention of heat nor in the symptomatology.

Of the several methods tried the most successful for the rapid cooling of the patients was that of submerging them in tubs of ice water.

An important question raised by these studies is why the patients cease to sweat and thereby fail to regulate their body temperature.

*Clinical and Chemical Observations on Forty-eight Men Suffering from Exposure to High Environmental Temperature.* By JOHN H. TALBOTT and (by invitation) D. B. DILL, Boston, Mass.

This communication is a summary of the data obtained on workmen who reported off duty following exposure to high temperatures. The men were employed in the steel mills at Youngstown, Ohio, and previous to the onset of symptoms were considered normal and healthy persons. After admission to the hospital one or more samples of blood were drawn from each patient for the following analyses; oxygen capacity, oxygen content, carbon dioxide content, cell volume, lactic acid, red and white cell count, sodium, potassium, calcium, chloride, phosphate, hydrogen ion concentration, total protein, osmotic pressure and nonprotein nitrogen. In urine samples the following were determined; albumin, sugar, acetone bodies, character of sediment, sodium, chloride, creatine, creatinine, and total nitrogen.

The clinical syndromes observed were placed in one of three categories. These are *heat cramps*, *heat prostration* and *heat pyrexia*. A diagnosis of heat cramps was made in 33 patients who complained of skeletal muscle pain. Analysis of the concentration of the constituents of the blood showed, among other changes, a diminished concentration of sodium and chloride, an increased concentration of hemoglobin and serum protein, and a normal osmotic pressure. In the urine there was a diminished concentration of chloride. During the hospital stay the gain in body weight was as great as 4 kgm.

A diagnosis of heat prostration was made in 12 patients who either collapsed at work or who believed that collapse was impending. On admission to the hospital the patients in this group had no characteristic physical findings, and the chemical studies of the blood and urine were essentially normal. There was no gain in body weight during the hospital stay, and it was presumed that the etiological mechanism was not associated with a loss of water and salt from the body.

Three patients were sent to the hospital because of unexplained fever. The pyrexia lasted 1, 3 and 21 days respectively. Except for the elevation of temperature, the clinical observations were negative and the chemical studies were normal. All of the patients lost weight in the hospital.

This study was an attempt to differentiate clinically and chemically the several syndromes associated with the breakdown of physiological processes in healthy persons. It was believed that more satisfactory data would be obtained with this objective than would be possible in groups of aged or infirm suffering from exposure to heat in large cities during the summer. The changes in the concentration of constituents of the body fluids in patients with *heat cramps* were characteristic of a depletion of body water and mineral salts. The pathogenesis of *heat prostration* is thought to be associated with transient circulatory collapse rather than an alteration of the chemical equilibrium of the body. The pathogenesis of *heat pyrexia* is assumed to be related to failure of the heat regulating mechanism.

*On the Mechanism of Migraine Headache and the Action of Ergotamine Tartrate.* By J. R. GRAHAM (by invitation) and H. G. WOLFF, New York, N. Y.

New light is thrown on the mechanism of migraine headache<sup>1</sup> through the analysis of the action of ergotamine tartrate. Investigation has been carried out as follows. The amplitude of pulsations in branches of the external and internal carotid as well as other arteries has been ascertained by means of optical levers and photography. In 14 patients with migraine headache, ergotamine tartrate (Sandoz) (0.3 to 0.5 mgm. intravenously) produced a decrease of as much as 80 per cent and an average of 50 per cent in the amplitude of the temporal artery pulsations. This effect persisted for an indefinite period (90 minutes or more). In persons suffering headache there was a simultaneous decrease in the intensity of the headache with the progressive decrease in the amplitude of the pulsations: if the amplitude decreased slowly, the intensity of the headache decreased slowly; if the amplitude decreased rapidly, the headache rapidly disappeared. Direct photography of the temporal artery taken before and after ergotamine injection showed actual constriction of the vessel. The middle meningeal artery also constricts.

On the other hand, ergotamine has little effect, if any, on the internal carotid and its branches, as indicated by

<sup>1</sup> For definition see Wolff, H. G., Arch. Neurol. and Psychiat., 1937, 37, 895.

the following data. The amplitude of the intracranial pulsations was measured through a needle in the lumbar subarachnoid space in 5 patients with migraine headaches, and in 28 controls without headache. Ergotamine sometimes produced a transitory decline followed by an actual increase in the amplitude of pulsations, but usually the effects were inconstant, of varying magnitude, and bore no relationship to the state of the headache. Photographs of the retinal arteries (branches of the internal carotid) before and after ergotamine revealed no change, though slight constriction in the veins was observed.

To show further that headache was associated with increased amplitude of cranial artery pulsations and relief associated with decreased amplitude, the following experiments were performed.

(a) Histamine phosphate (0.1 mgm.) was given intravenously to 3 patients just after the migraine headache had been abolished by ergotamine. The amplitude of temporal pulsations then rose to more than 250 per cent of the post-ergotamine level. With this increase in amplitude there was a return of severe headache.

(b) In one instance, histamine (0.05 mgm.) happened to produce hemicrania indistinguishable from the previously abolished migraine headache. Here the increase in amplitude of temporal artery pulsations was unilateral and on the affected side. In all instances, with the decline in the amplitude of the pulsations to the post-ergotamine level, the headache again disappeared.

(c) Similarly, in 11 subjects without headache, ergotamine tartrate given 15 minutes previously did not prevent the incidence of severe headache after histamine injection. The amplitude of intra- and extra-cranial pulsations increased 250 per cent during the period of most severe headache. These experiments show also that the action of ergotamine tartrate is not analgesic.

(d) In 6 subjects, pressure applied to the carotid or temporal arteries during the course of migraine headache decreased the amplitude of pulsations, and relieved the headache while pressure was applied.

(e) The administration of adrenaline (subcutaneous 0.012 cc. 1/1000 per kilo) during migraine headaches reduced the amplitude of temporal pulsations 30 per cent. This was accompanied by short-lived relief of the headache. With the return of pulsations to their original level within 8 minutes, the headache also returned to its initial severity. It was then abolished by ergotamine which caused a long lasting reduction of 75 per cent in the amplitude of temporal pulsations.

Despite the above described vasoconstriction after ergotamine tartrate, the peripheral circulation in the skin of the ear, cheek and hand (by radiometric determinations) showed inconsequential change, presumably due to the accompanying rise in blood pressure.

During maximum vasoconstriction of the temporal artery and at the onset and during the height of the headache relieving effect, reflex dilatation of the pupils on painful stimulation of the neck, as well as pilomotor reflexes were unimpaired. Moreover, the pressor responses to adrenaline were unchanged. These data indicate that after the given amount of ergotamine tartrate, smooth

muscle is capable of responding to sympathetic impulses and adrenaline.

It has also been shown, by placing a cannula in the temporal artery of a migraine patient, that increased intravascular pressure with distension of the artery was accompanied by pain. These data, and those described above, coupling the vasoconstrictor action of ergotamine tartrate with the relief from pain, lend support to the thesis that migraine headache is an expression of dysfunction of the cranial vascular bed, probably dilatation of certain branches of the external carotid artery.

*Antipneumococcus Rabbit Serum as a Therapeutic Agent in Lobar Pneumonia.* By F. L. HORSFALL, JR., K. GOODNER, C. M. MACLEOD and A. H. HARRIS, 2d (introduced by Oswald T. Avery), New York, N. Y.

Antipneumococcus rabbit serum has been found to possess numerous characteristics which make it theoretically a more advantageous therapeutic agent in lobar pneumonia than is immune horse serum. Of these the more significant are: (1) higher protective titer per milligram of specifically precipitable protein, (2) smaller size of antibody as determined by ultrafiltration and ultracentrifugation, (3) absence of prozone phenomenon when more than optimum amounts are used in protection test, (4) failure of lipids to inhibit protective action, (5) ease and certainty of immunization, and (6) high potency of unconcentrated serum.

Because of these indications, unconcentrated type specific antipneumococcus rabbit serum has been used in the treatment of 22 patients with pneumococcus pneumonia, 10 with Type I, 4 with Type II, 3 with Type VII, and 5 with Type VIII. Bacteremia was present in 12 of the 22, consolidation of two or more lobes in 7, bilateral consolidation in 3 and pleural exudate containing large numbers of pneumococci in 3.

The results of the clinical use of antipneumococcus rabbit serum have been characterized by: 1, a strikingly low mortality rate; 2, early occurrence of crisis; 3, absence of anaphylactoid reactions; 4, sterilization of infected pleural exudates; and 5, mildness of serum sickness. These results together with the ease and rapidity with which the serum can be administered, as well as the ability of rabbits to produce antisera against each of the various types of pneumococci suggest that antipneumococcus rabbit serum is a therapeutic agent of considerable merit.

*Allergy and Desensitization in Tuberculosis.* By H. S. WILLIS and (by invitation) C. E. WOODRUFF, Detroit, Mich.

When guinea pigs are infected with tubercle bacilli and become allergic, their allergic phenomena are associated with a demonstrable degree of immunity. It has recently been claimed that, when such animals are desensitized by the introduction of repeated doses of tuberculin, all allergic phenomena disappear but immunity remains. In these experiments groups of normal, allergic and desensitized guinea pigs were inoculated with virulent tu-



bercle bacilli and were sacrificed at varying intervals thereafter. From the spleens of these animals tubercle bacilli have been removed and their numbers determined. The work indicates definitely that tubercle bacilli spread over the body of the desensitized animal with distinctly more facility than they do over the allergic body and that the desensitized animal is actually not the same immune animal that it was during the days of its allergy.

Histological study of the spleen indicates development of widespread fibrosis as a result of the desensitization process.

*Studies on Anaphylaxis in the Isolated Heart.* By HERBERT B. WILCOX, JR. (by invitation), and E. COWLES ANDRUS, Baltimore, Md.

If the hearts of guinea pigs are isolated and perfused with Ringer-Locke solution, the introduction of horse serum into the normal heart via the perfusate produces no significant effect. In the heart removed from an animal previously sensitized to horse serum the introduction of antigen (0.005 cc.) provokes obvious, though evanescent, alterations in function: (a) Changes in cardiac rate; (b) Alterations in auriculoventricular conduction; (c) The development of ectopic rhythms; (d) Variations in the form of the electrocardiographic complexes; (e) Pronounced diminution in the rate of coronary flow.

The degree of reduction of the rate of coronary flow which always accompanies the other phenomena of cardiac anaphylaxis, and the fact that often it is the first change to appear, suggests that it may be of primary importance.

The effects of small amounts of histamine on such perfused hearts include: (a) Changes in cardiac rate; (b) The development of ectopic rhythms; (c) Variations in the form of the electrocardiographic complexes; (d) Pronounced diminution in the rate of coronary flow.

The presence of atropine in the perfusate in a concentration of 1:100,000 inhibits all the manifestations of cardiac anaphylaxis; but only the coronary-flow-diminishing power of histamine. The possibility of a functional inter-dependence existing between cardiac anaphylaxis, the coronary vascular bed, and perhaps the production of an histamine-like substance is suggested.

*The Influence of Diet on the Course of Nephrototoxic Nephritis in Rats.* By LEE E. FARR and JOSEPH E. SMADEL (introduced by Homer F. Swift), New York, N. Y.

The renal injury, induced in rats by anti-kidney serum, provides a tool for testing the rôle of diet in nephritis, a question that has been long under discussion in the treatment of Bright's Disease.

Forty-eight young rats were fed a purified diet for a period of one month, while renal function studies, plasma protein and hemoglobin determinations and urinalyses were made. Then severe nephritis was induced uniformly in all the animals by intravenous injections of nephrotoxic serum given on three successive days. The animals were divided into three comparable groups and

fed different isocaloric diets. Each diet contained 27 per cent fat, 4 per cent salt mixture, and vitamins. They differed as follows: Diet 1, 64 per cent carbohydrate and 5 per cent protein; Diet 2, 51 per cent carbohydrate and 18 per cent protein; Diet 3, 29 per cent carbohydrate and 40 per cent protein. Diet 2 was used during the control period. Observations made during the control period were continued at three-week intervals for eleven months following injection.

The course of the nephritis in all three groups was parallel for one month following injection. During the second month evidences of nephritis markedly diminished or disappeared in all but two animals on Diet 1. These two died in the fifth month without renal failure. All other animals in this group survived, and at the end of the experiment all but three had normal urine. Every animal fed Diet 3 showed progressive nephritis, and thirteen died of apparent renal failure during the experiment; the remaining three were in the terminal phases of the disease when sacrificed. Of the animals fed Diet 2, one recovered in the second month, nine died of apparent renal failure during the experiment, and the remaining six had abnormal urine findings throughout. It is concluded that: 1. Chronic progressive nephritis follows a *single* insult to the kidney, under certain conditions. 2. The course of nephrotoxic nephritis in rats can be markedly influenced by diet.

*Hemoglobin Nephropathy; A Pathological Study in Man and the Dog.* By ELMER L. DEGOWIN and EMERY D. WARNER (introduced by Horace M. Korns), Iowa City, Iowa.

The tissues of 8 humans dying of transfusion anuria and one dying from quinine hemoglobinuria were studied. It is evident from a study of these and a comparison of other cases reported in the literature that two distinct types of nephropathy occur. In one, there is little evidence of tubular obstruction but there are varying degrees of necrosis, particularly in the epithelium of the proximal convoluted tubules. In the other type, epithelial degeneration is minimal while the distal portions of Henle's loops are plugged with hemoglobin precipitate and crystals while the proximal lumina are dilated. The glomeruli are not damaged in either case.

Dogs were transfused with hemoglobin solutions. When the urine was alkaline the kidneys were not affected. When the urine was acid, however, they developed uremia and died. The recurring portions of Henle's loops were plugged with hemoglobin pigment and crystals while the proximal lumina were dilated. The tubular epithelium showed little necrosis. In one dog subjected to the same procedure, however, there was tubular necrosis but no pigment obstruction.

We conclude that most of the dogs and some of the humans developed renal insufficiency because of the precipitation of hemoglobin in an acid medium producing obstruction of the tubules. This explanation does not account for some of the human cases although the clinical course is similar in all.

*The Urinary Excretion of Androgenic and Estragenic Substances in Normal Men and Women and in Hypogonadism, Gynecomastia and Virilism.* By T. F. GALLAGHER, A. T. KENYON, D. H. PETERSON, R. I. DORFMAN and F. C. KOCH (introduced by O. H. Robertson), Chicago, Ill.

Benzene extracts of hydrolyzed urine were assayed for androgenic properties upon the capon's comb and for estrogenic properties upon adult spayed rats. The values given below are based on the two hour hydrolysis.

Four normal young men, studied for six weeks, excreted an average of 40 international androgen units per day (range 13 to 79), and an average of the equivalent of 10 gamma of theelin per day (range 2 to 29), with no conclusive evidence of cyclic variation.

Four normal young women, studied for four weeks, excreted an average of 28 international androgen units per day (range 13 to 51), and an average of the equivalent of 25 gamma of theelin per day (range 4 to 60). The cyclic increase in the estrogen output during the intermenstruum was unaccompanied by alteration in the androgen output.

Eight male hypogonads excreted about a third of the normal androgenic and estrogenic material. Four men with gynecomastia showed no conclusive changes in the amount of excreted hormones. Twenty women with virilism showed normal values as a rule, slightly high values for the androgenic material on occasion, and in one instance (adrenal cortical carcinoma) the high value of 480 international androgen units. The urine with the high values showed spectrographic properties unlike androsterone and like certain adrenal cortical derivatives.

*Evidence for the Existence of Two Factors Necessary for the Successful Treatment of Pellagra or Experimental Canine Black Tongue.* By JULIAN M. RUFFIN, ELBERT L. PERSONS and H. I. HARVEY (by invitation), and DAVID T. SMITH, Durham, N. C.

Patients with acute pellagra were hospitalized and fed a diet adequate in all respects except the pellagra preventive factor of Goldberger. The tests were limited to those patients who showed increasing symptoms while subsisting on the basic diet and those whose symptoms were reactivated by exposure to graded doses of sunlight. The criteria of a successful remission were (1) prompt disappearance of all symptoms, (2) marked increase in appetite, and (3) failure of maximum doses of sunlight to reactivate the disease.

Rapid and complete recovery was noted in 20 of 22 patients receiving an aqueous extract of 675 grams of liver daily. "Solution liver extract—Valentine (N. N. R.)."

Twenty-three patients were treated by parenteral injections of various liver extracts potent for pernicious anemia. Total doses derived from 400 to 6,750 grams of liver gave prompt improvement in the mouth and tongue symptoms, but there was no improvement in appetite, no constant effect on the diarrhea and in most instances relapse occurred after exposure to sunlight.

One patient was given orally the extract for parenteral use derived from 700 grams of liver daily for 6 days without improvement.

The liver residue discarded in the process of manufacturing the parenteral extract was ineffective in 5 patients who received total doses derived from 7,680 to 9,600 grams of liver. A dose derived from 28,800 grams was effective in one case of pellagra but this same massive dose was also effective in 3 cases of pernicious anemia and one of sprue.

The combination of the partially effective dose of the parenteral extract (derived from 700 grams) and the ineffective dose of residue (derived from 9,600 grams) produced an immediate and dramatic remission in 4 cases of pellagra.

Black tongue was produced in dogs by feeding a modification of Goldberger's black-tongue producing diet number 123. The treatment was limited to a ten day period. The criteria for a good result were (1) cure of the mouth lesion, (2) restoration of the appetite, and (3) a prompt gain in weight which must be maintained for 10 days after the treatment is stopped.

An aqueous extract of whole liver, "solution liver extract—Valentine (N. N. R.)," was effective in 3 dogs receiving a total dose derived from 3,670 grams of liver.

In 5 dogs parenteral injection of pernicious anemia liver extract derived from 300 grams of liver improved the mouth temporarily but had no other effect.

Five dogs received 3,000 grams of the parenteral liver with marked improvement in the mouth but unsatisfactory weight gain and a relapse of black tongue after 22 days.

Eleven dogs received the residue derived from 1,000 grams of liver without improvement. One dog made a slow improvement after receiving the residue derived from 2,000 grams and 3 dogs made a satisfactory recovery when the dose was raised to 3,000 grams. Even better results were obtained in 14 dogs when the 3,000 gram dose was supplemented with 300 of the parenteral.

When the ineffective dose of parenteral extract (derived from 300 grams) was given with the ineffective dose of the residue (derived from 1,000 grams) to four dogs, dramatic improvement resulted which differed from our best results only in the shorter length of time which intervened before the next attack of black tongue.

From these observations it would seem that there are two factors in liver which are necessary for the successful treatment of pellagra in man and experimental black tongue in dogs.

*The Relation of Vitamin B to Serum Proteins and to Edema.* By HENRY FIELD, JR., Ann Arbor, Mich.

Nutritional edema has been commonly considered to be the result of deficiency in protein intake. In 1935 Elsom reported studies on two patients with decreased serum protein and edema while on a diet adequate in protein but deficient in vitamin B.



We have observed cases with edema and lowered serum proteins despite diets apparently adequate in both protein and vitamin who had no apparent cardiac or renal cause therefor. Relief has followed supplements of vitamin B complex. These include cases of cirrhosis of the liver, one of whom has been maintained edema free and with nearly normal serum proteins for one year despite increasing impairment of liver function by other evidence. In another, who has recently developed evidence of hepatic insufficiency after two years of treatment, edema and lowered plasma proteins have recurred three times when the vitamin supplement has been neglected and have been relieved each time by its resumption.

One case of amyloidosis developed edema four weeks after urine specimens were known to be normal and at a time when he had mild proteinuria. Edema disappeared and serum proteins increased despite greatly increased proteinuria. Another patient had had a partial gastrectomy for duodenal ulcer and a jejunostomy for intestinal obstruction. In three of these patients edema has disappeared before the serum proteins reached the "critical level." One case of nutritional edema with normal serum proteins has been promptly relieved by vitamin B supplements.

It is suggested that vitamin B complex is a factor in the maintenance of serum proteins and that an increased intake may be beneficial when they are lowered in organic disease. It is also suggested that it is a factor in production of edema aside from its relation to serum proteins.

*Positive Pressure Respiration and its Application to the Treatment of Acute Pulmonary Edema and Respiratory Obstruction.* By ALVAN L. BARACH and (by invitation) JOHN MARTIN and MORRIS ECKMAN, New York, N. Y.

Evidence is presented which supports the concept that obstructive dyspnea produces pulmonary congestion, edema and emphysema because of the pathologically increased negative pressure within the chest during inspiration. The application of moderate positive pressures to inhalation therapy reduces the negative intrapleural pressure, relieves the dyspnea and tends to prevent pathological changes in the lungs.

In various types of experimental and clinical acute pulmonary edema the most important causal factor appears to be the negative pressure in the lungs during the inspiratory phase of respiration, since a suction action is exerted on pulmonary capillaries which may already have a tendency to ooze serum either because of engorgement or increased capillary permeability. Inhalation of air, oxygen or helium mixtures is followed by a clearance of pulmonary edema, the mechanism of which appears to be the lowering of the pathologically increased negative pressure within the chest.

The effects of positive pressure respiration on circulatory function in normals, patients with cardiac insufficiency and patients with asthma were presented.

*The Deposition of Radium Salts in Bones, and Their Relation to Calcium Metabolism.* By J. ALFRED CALHOUN and JOSEPH C. AUB, Boston, Mass.

Radioactive lead, a disintegration product of the gas Radon, was used to demonstrate the effects of acute poisoning. A small amount of this substance, given intravenously to three dogs, caused death in from ten to twelve days. Because of the presence of a radioactive salt, the bones were self-photographic, and it was shown that most of the radioactive lead had been deposited in the trabeculae. Analyses, done by very delicate physico-chemical methods (sensitive to 0.0001 mgm.), showed that the trabeculae contained an average of eleven times as much radioactive lead as the cortical bones.

Bones and teeth from two patients demonstrate the chronic effects of radioactive materials. A self-photograph of a tooth from a living patient, who has been a radium dial painter for fourteen years, shows radium deposited deeply in the tooth. Thus, the area of inorganic salt deposit in teeth has been established. Self-photographs of bones secured at autopsy from a patient who received "Radium water" and who died of a neoplasm of the bones of the knee, demonstrate the position of heavy metals in bones long after exposure has ceased. Analyses of these bones show practically an even distribution of radium between the trabeculae and cortex.

Therefore, it is clear that therapy should be more effective early, for at that time heavy metals are localized in regions where they can be more readily mobilized.

*The Use of the Cathode-ray Oscillograph in the Study of the Monocardiogram.* By FRANK N. WILSON, FRANKLIN D. JOHNSTON and PAUL S. BARKER, Ann Arbor, Mich.

The concept of a single figure which represents the direction of the electrical axis and the magnitude of the cardiac potential at every instant during the cardiac cycle is not new. Mann has referred to this figure as the monocardiogram. Such curves can be constructed from measurements made on any two of the three standard leads recorded simultaneously, but the process is too laborious for use in the analysis of a large number of electrocardiograms. When conventional Lead I and the potential variations of the left leg ( $V_F$ ) are amplified to the required degree and impressed upon the plates of a cathode-ray tube, the former upon the horizontal and the latter upon the vertical plates, the monocardiogram is inscribed on the end of the tube by the luminous spot produced by the electron beam and may be easily photographed.

We have taken a considerable number of monocardiograms in this way, using both normal individuals and patients with various electrocardiographic abnormalities as subjects.

The figures obtained are closed loops of varying size and shape. The spot may traverse the loop in a clockwise or counterclockwise direction. Irregularities in its movement, or a reversal in the direction of its motion, occurs in cases in which conspicuous notching of the

QRS group is present. The orientation of the loop with reference to the zero position of the spot depends on the position of the mean electrical axis.

It is hoped that the monocardigram may give information of value in the analysis of the electrocardiogram by making it possible to distinguish between curves which have a similar appearance but are fundamentally different with respect to the origin of their components. The method makes it possible to convert the three curves of the standard leads into a single curve and this greatly simplifies the analysis of the form of the electrocardiographic deflections.

*Metabolic Studies on Idiopathic Steatorrhea.* By SAMUEL H. BASSETT and E. HENRY KEUTMANN (introduced by Wm. S. McCann), Rochester, N. Y.

Lipid, nitrogen, calcium and phosphorus balances have been determined in three cases of idiopathic steatorrhea in an attempt to evaluate various therapeutic procedures. Prolonged administration of large doses of liver extract intramuscularly was found ineffective in all cases. Replacing starches and sucrose in the diet by addition of equivalent amounts of dextrose and ripe banana, while the quantity and source of fat remained constant, did not cause improvement in the steatorrhea. Absorption of fatty acids was not improved by glycerophosphate or ox bile given orally.

Two patients showed the expected decrease in steatorrhea when the intake of fat was sharply restricted. In one of these, defective absorption of calcium was not improved by practically complete removal of fat from the diet. In a third patient with a relatively mild steatorrhea, the fatty acids of the feces could be reduced fifty per cent by shifting from normal to a low calcium intake. It is believed that the steatorrhea in this last patient was caused in part at least by defective absorption of calcium, failure to absorb fatty acids with the usual completeness being due to formation of insoluble calcium soaps in the small intestine.

#### READ BY TITLE

*Factors Controlling the Behavior of Glucose in the Human Stomach and Small Intestine.* By W. OSLER ABBOTT, WALTER G. KARR (by invitation) and T. GRIER MILLER, Philadelphia, Pa.

The factors governing the behavior of glucose in the normal human digestive tract have been studied. Evidence has been secured to show that a glucose solution, even of 50 per cent concentration, begins to leave the stomach within two minutes, but that the concentration of the solution as it enters the duodenum may be reduced to 15 per cent or less. The glucose may in turn enter the jejunum within two minutes but when its concentration usually is only 3 to 5 per cent. The rapid reduction in the sugar content of the ingested solution from 50 per cent to 5 per cent within less than five minutes and during its passage along a scant two feet of alimentary canal is accomplished by dilution and by selective absorption of glucose from the fluid of the duodenal

contents. The capacity of the diluting mechanism has been tested and, excluding the bile and pancreatic juice, the fluid derived from the duodenal mucosa alone has been found sufficient to double the volume of a 25 cc. sample of a 15 per cent solution in four minutes. The response of the duodenum to glucose has been compared to that of the jejunum and ileum, and the changes in the electrolyte concentration and the osmotic pressure of the intestinal contents have been determined.

*Photometric Studies of Visual Adaptation in Relation to Mild Vitamin A Deficiency in Adults.* By JOHN B. YOUNG and (by invitation) MARVIN B. CORLETTE, HELEN FRANK and MILDRED G. CORLETTE, Nashville, Tenn.

Using an improved type of visual photometer we have studied the dark adaptations of a group of 54 presumably healthy adults and a group of 50 adult outpatients of our clinic. Eighty-three per cent of the former had initial recovery readings, after blanching, of 0.7 millifoot candles or less, while 50 per cent of the patient group showed poorer readings than this. Practically all subjects in both groups who had relatively poor dark adaptations and who were given large amounts of vitamin A for 4 to 6 weeks showed definite improvement and came within the limit of 0.7 millifoot candles. These findings have led us to propose 0.7 millifoot candles in the initial recovery reading as a tentative limit of normal dark adaptation in adults. Normal subjects with good adaptations were improved only very slightly after similar treatment showing that they were already at their optimum levels of dark adaptation, and vitamin A nutrition.

*Clinical Results with the Elsberg Olfactory Test.* By ALEXANDRA ADLER and KNOX H. FINLEY (introduced by Tracy J. Putman), Boston, Mass.

Elsberg's investigations have opened a wide field for physiological and clinical studies on olfaction.

For clinical purposes his most important methods are:

1. The "M. I. O." (minimal identifiable odor) expressing the minimum amount of air saturated with odor necessary for recognition.
2. "Olfactory fatigue," which is the interval of rest after an odor fails to be perceived before it can be perceived again.

Elsberg found an increased M. I. O. in cases of brain tumor producing direct pressure on the olfactory tract and an increased olfactory fatigue with tumors within the brain substance.

In 7 verified pituitary tumors with signs and supra-orbital tumors respectively we found the M. I. O. normal in 4 and increased in 3. The olfactory fatigue was normal in all 7. In 16 verified cerebral tumors without direct pressure on the olfactory tract we found the M. I. O. increased in 3 and normal in 13. Olfactory fatigue was increased in one, undeterminable in one because of the high M. I. O. and normal in 14.

In conclusion the Elsberg olfactory test enables us to determine quantitatively the olfactory function. In a few of our cases with direct pressure on the olfactory tract the M. I. O. was increased suggesting that this part of the test may be of diagnostic help in such cases. In respect to intracerebral growths the occurrence of increased olfactory fatigue seems to be too rare (1 out of 16) to be of practical clinical value.

*Hematopoietic Principle in the Diseased Human Liver.*

By LEON SCHIFF and (by invitation) MURRAY L. RICH and STANLEY D. SIMON, Cincinnati, Ohio.

In an attempt to elucidate the mechanism of macrocytic anemia in liver disease, extracts made of livers obtained from two cases of hepatic cirrhosis with associated macrocytic anemia were administered intramuscularly to patients with pernicious anemia in relapse. Positive hematopoietic responses were obtained. Similarly prepared extracts made from cases of acute yellow atrophy, toxic hepatitis, chronic passive congestion and malignant neoplasm of the liver also yielded positive responses.

The results indicate that the severely diseased liver may store the antianemic principle and that the macrocytic anemia of liver disease cannot necessarily be ascribed to failure of such storage.

*The Production of Signs and Symptoms of Toxemia of Pregnancy by the Administration of Sodium Salts to Pregnant Women with Hypoproteinemia.* By MAURICE B. STRAUSS, Boston, Mass.

Twenty-four women have been studied in the last trimester of pregnancy. Eight women with known pre-existing arterial hypertension without exacerbation in pregnancy and with essentially normal plasma proteins were given daily 6.3 grams of sodium (either as 16 grams of the chloride or 23 grams of the bicarbonate) for one week. Water was allowed ad libitum. Slight increments of weight unassociated with blood pressure changes or other signs or symptoms occurred.

Ten women with hypoproteinemia and arterial hypertension in the last trimester of pregnancy were given similar amounts of sodium daily. Eight of these women had no pre-existing arterial or renal disease. Weight gains of from 3.3 to 7.7 per cent together with gross edema occurred. The arterial blood pressure, particularly the diastolic, rose significantly. In 5 women increasing albuminuria was noted, and in 3 symptoms of pre-eclampsia became so alarming that the administration of sodium had to be discontinued within 4 days. Six women with hypoproteinemia were observed under identical conditions, but without the administration of sodium salts, as a control of the above observations. No gain in weight, edema, elevation of the blood pressure, or other symptoms occurred. The administration of a high protein diet to these women resulted in weight losses of from 2.9 to 4.1 per cent and the subsidence of symptoms.

The conclusion is drawn that the signs and symptoms of toxemia of pregnancy in the patients studied resulted from water retention induced by sodium salts in the presence of hypoproteinemia.

*Studies in Pernicious Anemia. Extirpation of "Intrinsic Factor" Sources.* By ARTHUR GEIGER, LOUIS GOODMAN and LOUIE N. CLAIBORN (introduced by Francis G. Blake), New Haven, Conn.

Experiments on swine designed to elucidate further the rôle of gastro-intestinal factors in pernicious anemia have been carried into the third year. The main sources of the "intrinsic factor" have been eliminated by total gastrectomy, isolation of the pylorus, duodenectomy, and gastrectomy plus duodenectomy.

Thirty-six young swine were used. Growth and development were normal in all animals surviving simple gastrectomy. Only a mild hypochromic anemia resulted during the first postoperative year despite repeated pregnancies. One sow, now 30 months agastric, has in the past 6 months developed a striking and steadily progressing macrocytic and hyperchromic red cell morphology. Bone marrow studies are in preparation. Spinal cord lesions have thus far appeared in only one animal. Clinical assay of the livers from agastric animals revealed appreciable loss of reticulocytogenic power in 2 months, marked depletion in 6 months, and complete absence of antianemic potency in 18 months. Gastrectomy plus duodenectomy, and duodenectomy alone resulted in considerable disappearance of liver potency within several months. The differentiation between a hormonal and enzymatic nature of the antianemic principle is being sought by assay of livers of newborn pigs obtained from agastric and from normal sows.

*Medical Treatment of Hyperthyroidism with a High Fat Diet.* By S. SOSKIN and (by invitation) I. A. MIRSKY, Chicago, Ill.

The present adequacy of surgery in hyperthyroidism has left little opportunity for the observation of medical treatment in severe cases. A patient who absolutely refused operation offered us such an opportunity. The results are recorded in order that others may be able to compare their observations as occasion arises.

The rationale of our treatment was based on several reports that diet may influence the hyperthyroid state or the action of administered thyroxin. More specifically, Loumos and also Seoz have shown that the feeding of certain fats may inhibit the activity of thyroxin in experimental animals. It seemed possible that the hypolipemia in hyperthyroidism might be playing a primary rather than a secondary rôle.

A woman, age 36, was admitted to the Max Pam Unit, Michael Reese Hospital, on June 10, 1935, four months after the advent of an acute hyperthyroidism. She complained of nervousness, palpitations, loss of weight and oligomenorrhea. She showed thyroid enlargement, lid lag, tremor, pulse rate of 116, blood pressure 140/74, basal metabolic rate plus 62.9, blood cholesterol from 147 to 176, and she weighed 110 pounds. On a diet of P. 90 F. 230 CHO. 90, with 3 grams of cholesterol per day added to the butter ration for about 3 weeks, she soon showed remarkable improvement. No iodine was given at any time. She was discharged on October 15, 1935,

on the above diet, and has been observed at weekly intervals to date. At present there are no signs or symptoms of hyperthyroidism. Pulse rate is 70 to 80, blood pressure 120/70, basal metabolic rate minus 6 to plus 13, blood cholesterol over 200 mgm. per cent and weight 140 pounds.

*Studies of Blood Formation in the Fetus and Newborn. IV.*<sup>1</sup> By M. M. WINTROBE and (by invitation) DEAN A. CLARK, Baltimore, Md., WILLIAM TRAGER, Princeton, N. J., and LEWIS DANZIGER, Baltimore, Md.

Whereas extracts for parenteral injection made from the livers of pig fetuses showed no antianemic potency in cases of pernicious anemia, desiccated liver of the last third of the gestation period, administered orally, caused a slight reticulocyte response in two out of three cases. However, the erythrocyte count did not rise and desiccated adult liver caused a much more striking response. Desiccated placenta showed no potency whatever. In assays for the growth promoting factor needed by mosquito larvae, the desiccated fetal livers were found to be about half as potent as desiccated adult liver. This factor was present in desiccated placenta but the quantity was small as compared with that found in adult liver.

Desiccated fetal stomach, on the other hand, appears to contain significant amounts of the antianemic substance effective in pernicious anemia, although the quantity may be less than in desiccated adult stomach. A concentrate of milk vitamins has been shown to contain "extrinsic factor." Extracts of the livers of stillborn pigs administered parenterally, have shown no potency but the limited observations made to date indicate that antianemic substance is found in increasingly greater concentration in the liver as age advances.

*The Site of the Interaction of Extrinsic and Intrinsic Factors in Pernicious Anemia.* By WILLIAM B. CASTLE and (by invitation) R. W. HEINLE, Boston, Mass.

Convincing evidence is lacking for any specific action *in vitro* of normal human gastric juice (intrinsic factor) upon beef muscle (extrinsic factor) responsible for the observed increased blood production and clinical improvement in pernicious anemia. Indeed, since neutral mixtures are effective, the essential interaction between these factors may take place only after their absorption from the alimentary tract. The purpose of the present observations was therefore to discover whether any essential interaction between beef muscle and gastric juice occurs within the alimentary tract, and, if so, whether such interaction results in the production of the active principle of the normal mammalian liver. During successive 10-day periods of the daily administration of test mixtures reticulocyte and red blood cell counts were made

upon 8 patients with Addisonian pernicious anemia while on controlled dietary regimes.

The daily administration of mixtures of beef muscle and gastric juice incubated for 6 hours and administered at an acid reaction (pH 1.8 or 2.5) produced negative results. However, positive effects upon blood formation were observed if, after such incubation, the mixture was administered at a reaction of pH 5 to 7. Control observations showed that a mixture of beef muscle and gastric juice incubated with pepsin for 12 hours at pH 1.8 was effective upon administration after neutralization. The failure of the unneutralized mixtures was therefore apparently not due merely to the prolongation during an *in vivo* period of a destructive process begun during the *in vitro* acid incubation. Thus, the strongly acid reaction seemingly provided an environment unsuitable for the essential interaction of the beef muscle and gastric juice. This unfavorable effect must have occurred *in vitro* or within the intestinal tract rather than parenterally.

The activity of mixtures of beef muscle and gastric juice, with or without the serial addition of normal human duodenal contents or hogs' duodenal and small intestinal mucosa after 2-hour incubation at pH 7, was destroyed by temperatures (40° to 100° C.) not destructive of the activity of aqueous solutions of the liver fraction "G" of Cohn, Minot and their associates. Therefore, contrary to the assertion of Klein and Wilkinson,<sup>1</sup> the formation of the thermostable active principle of liver could not be demonstrated *in vitro* under the above conditions.

As shown above, the active principle of liver is apparently not formed from beef muscle *in vitro* by serial contact with gastric, duodenal or intestinal secretions. It is therefore probable that, although an essential interaction between beef muscle and normal human gastric juice occurs within the alimentary tract, it involves only a preliminary step in the formation of the active principle of liver.

*Observations on the Action of Aminophyllin on the Intrathecal and Venous Pressures and on the Bronchi as a Possible Mechanism for Its Beneficial Effect on Cheyne-Stokes Breathing, Dyspnea, and Paroxysmal Dyspnea.* By JAMES A. GREENE, and (by invitation) W. D. PAUL and A. E. FELLER, Iowa City, Iowa.

The mechanism by which theophyllin-ethylenc-diamine ameliorates cardiac dyspnea and converts Cheyne-Stokes breathing to a regular rhythm is not known and has been the object of this investigation. These effects of the drug are attributed to an improvement in cerebral circulation.

The effect of intravenous administration of the drug upon the intrathecal and venous pressures measured both separately and simultaneously has been observed. The intrathecal and venous pressures decrease almost simul-

<sup>1</sup> This investigation has been made with the assistance of grants from The National Research Council and The Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

<sup>2</sup> Klein, L., and Wilkinson, J. F., *Biochem. J.*, 1934, 28, 1684.

taneously with the improvement of respiratory distress or change of periodic breathing to a regular rhythm. Amyl nitrite and nitroglycerine, on the other hand, increase the intrathecal and venous pressures and do not alter the respirations.

Bronchial obstruction may be relieved, if it is present, as evidenced by the increase in vital capacity and subjective relief when the drug is administered to patients during an acute attack of bronchial asthma of allergic etiology.

*Studies on the Anemia of Chronic Glomerulonephritis and Its Relationship to Gastric Acidity.* By S. R. TOWNSEND, E. MASSIE and R. H. LYONS (introduced by J. P. O'Hare), Boston, Mass.

Forty-eight cases of chronic glomerulonephritis were studied in relation to the anemia, degree of nitrogen retention and gastric acidity. In addition, histological studies were made of rib marrow and sections of stomach in thirty-one cases of glomerulonephritis.

The result of our observations indicate that the anemia was of the normocytic type, the red cell being normal in size with the hemoglobin content of the cell slightly decreased. The anemia became manifest with the development of renal insufficiency and increased with the degree of nitrogen retention. As the anemia, renal insufficiency and retention of nitrogen became more marked, gastric acidity was found diminished. Absolute achlorhydria was discovered when acidosis became evident and the blood CO<sub>2</sub> content fell below thirty volumes per cent.

Our investigations, along with those of others, lead us to feel that the diminished to absent gastric acidity plays an important rôle in the improper digestion and absorption of ingested food and iron and can account, in part at least, for the persistence of the anemia and its lack of response to therapy.

Studies of the bone marrow do not support the common theory of lack of active blood-forming tissue as the etiological factor in the production of the anemia since the postmortem rib marrow is in a normal or hyperplastic state. Studies of sections of stomach did not reveal any histopathological explanation for a diminution of the gastric acidity, although many cases died in acidosis and uremia.

*The Effect of Alcohol on the Water and Electrolyte Balances in Man.* By WILLIAM M. NICHOLSON and HAYWOOD M. TAYLOR (introduced by D. T. Smith), Durham, N. C.

The effects of alcohol on the electrolytes and water balances have been studied in normal, healthy, adult male volunteers. The subjects were placed on a constant diet, aliquots of which were analyzed for their sodium, potassium, chloride, nitrogen and water contents. The day was divided into eight hour periods. Urine and stool collections were made for these periods. At the end of three days on the diet the subjects were given alcohol in sufficient quantities to produce intoxication.

The sodium, potassium, chloride, and nitrogen content of the urine was determined, and complete electrolyte

studies were carried out upon the blood. Plasma volumes were estimated by the dye method of Gregersen. Estimations of the alcohol content of the blood were made to see if any correlation could be demonstrated between alcohol content and changes in the electrolytes.

It was found that during the period of intoxication there was a marked retention of the urine potassium, which was reflected in an increase of the plasma potassium concentration. To a lesser degree there was a retention of sodium chloride and water. An increase in plasma volume and in body weight was also noted. No constant change was found in the blood sugar; there was, however, a slight decrease in the carbon dioxide content and an increase in lactic acid.

From these data it is concluded that in acute alcoholism in man there is an increase in the circulating blood volume. Since the increase in the plasma potassium concentration is not great enough to explain the total potassium retained, it would appear that the intracellular fluid is also increased. It is suggested that some of the sequelae of alcoholic intoxication are due to an excess of potassium.

*The Effect of Certain Specific Dietary Factors on the Circulation in Patients with Heart Disease.* By SAMUEL H. PROGER and (by invitation) HEINZ MAGENDANTZ, Boston, Mass.

In the past we have reported the results of observations on the effects of general dietary restriction on the circulation in patients with heart failure. On such a regime there is a disturbance of balance in sodium, nitrogen and water. This report deals with an attempt to isolate the effects of these three factors. The results in general have been as follows:

(1) There is under certain circumstances a definite relationship between water and oxygen metabolism, the basal metabolic rate falling as the 24-hour level of water exchange is decreased and vice versa. While there is at times evidence that fluid restriction may have beneficial effects on the circulation, large quantities do not appear to alter significantly the dynamics of the circulation.

(2) When other factors are controlled, a reduction of protein intake with the development of a negative nitrogen balance is associated with a lowering of the basal metabolic rate. This is not constant, and changes in nitrogen balance produce relatively little effect on the state of the circulation of patients with cardiac insufficiency.

(3) After heart failure seems well overcome and a steady state established, a total of 16 grams of sodium chloride in the diet with a retention of 4 to 6 grams daily will, in two to four days, produce striking evidence of heart failure as manifested by increased venous pressure, diminished vital capacity, increased heart rate, elevated blood pressure, enlargement of the liver and edema. The effect upon the liver appears disproportionately great.

The sodium retention in these experiments is of about the same magnitude as in the acute infections and may act in the latter state as an important contributing factor

in the precipitation of heart failure in patients with organic disease.

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Three selected recurrent pellagrins were admitted to the Tennessee Coal, Iron and Railway Hospital of Ensley, Alabama, and given a diet rich in pellagra-preventive foods and calculated to meet basal energy requirements. Despite the fact that each patient was in good health at the time of admission and was observed to have eaten the required amount of the prescribed diet at each meal, he developed symptoms of pellagra within a month. These symptoms disappeared after the diet was increased in amount and supplemented with large amounts of powdered brewers' yeast.

The findings of this study in no way contradict the statement that pellagra is a dietary deficiency disease, but do point out that an occasional person at least, probably because he is unable to utilize properly the essential substances present in food, is not protected by a diet which prevents other people from developing pellagra.

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The respiratory rate, depth of inspiration and expiration, total ventilation per minute, oxygen consumption and the type of breathing, all were noted. In fourteen of the psychoneurotic patients, pleasant ideas were associated with a decrease in rate of respiration, a decrease in amplitude, a decrease in the total minute ventilation, and a marked tendency toward rounding of the spiro-

gram at the end of the expiration phase. During the period of unpleasant ideas in fourteen of these patients there was an increase in the respiratory rate, an increase in total ventilation, and a marked tendency to the formation of more acute angles on the spirogram at the end of the expiration. During the relaxed period the type of respiratory curve approximated that of the pleasant period. There was no marked change in oxygen consumption during any of the periods.

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The results were such as to exclude bile as a specific treatment for the disease. However, many of the patients obtained definite relief, and of 27 who had seasonal attacks 18 were kept symptomless. Observations



on these patients suggest that bile has a beneficial effect which does not seem readily explicable by a lowering of gastric acidity.

*The Use of Ergonovine in Migraine.* By WILLIAM G. LENNOX, Boston, Mass.

Ergonovine is the recently isolated alkaloid of ergot. Its effect in aborting headaches of the migraine type has been tested in a large group of patients. Injection of ergonovine is less effective than ergotamine tartrate in stopping individual headaches, but is preferred by some patients because its use is followed by less nausea and vomiting. The action of the two drugs on blood pressure, pulse and blood gases has been studied and compared.

*A Study of the Bactericidal Properties of the Synovial Fluid and Blood of Patients with Gonococcal Arthritis.*

By WESLEY W. SPINK (by invitation) and CHESTER S. KEEFER, Boston, Mass.

For several years we have been studying the various properties of the synovial fluid obtained from patients with gonococcal arthritis. It was pointed out several years ago that bacteriological study of samples of synovial fluid from patients with this type of arthritis failed to demonstrate the presence of gonococci in all cases. To determine whether this might be explained in part by differences in the bactericidal activity of the synovial fluid, the following investigation was carried out. Bactericidal tests were done on the blood and synovial fluid of a group of patients with gonococcal arthritis; 4 had infected fluids and 10 were uninfected. Four other specimens of synovial fluid which were obtained from other types of arthritis were studied and used as controls.

It was found that when the fluids were infected there was a wide difference in the bactericidal power of the synovial fluid and whole blood. When the fluid was sterile the bactericidal content of the blood and synovial fluid was the same.

The complement titer of the synovial fluid was found to be the same or slightly lower than that of the blood, and the bactericidal action was abolished by heating at 56° C. for 2 hours. The bactericidal action of synovial fluid could be enhanced by the addition of antigonococcal immune serum.

The evidence so far indicates that the difference between infected and sterile samples of synovial fluid from patients with gonococcal arthritis is due in part to the presence of antibodies in those which are sterile and their absence in those which are infected. An investigation of other factors is being pursued at present.

*Bacterial Type Transformation.* By HOBART A. REIMANN, Philadelphia, Pa.

A strain of *M. tetragenus* isolated from the blood of a patient with purulent arthritis and meningitis produced typical white colonies on agar. In a study of the microbic dissociation of the organism over a period of

two years, four major variant forms of the original "White" form were isolated. The variant colonies were characterized by distinctive pigments and were called pink, yellow, pink-yellow and brown. Since each of the variants were immunologically specific, and since the component M, S and R culture phases of each of these variants were subsequently isolated, they were regarded as specific types. Spontaneous transformation from one type into another in broth, on agar or *in vivo* in mice was frequently noted.

Further studies of the characteristics of differential growth of the five types indicated that the S form of the white type, originally isolated from the patient, was peculiarly fitted to survive *in vivo* as regards optimal growth in certain ranges of temperature and pH concentration *in vitro*. This observation suggested that in the process of bacterial variation of certain so-called saprophytes, variant types which are especially endowed with capabilities to exist *in vivo* under certain conditions, may appear spontaneously. Such variant types may be regarded as possessing potential "virulence." Further observations and evidence that a similar phenomenon occurs among other bacteria will throw considerable light on certain obscure problems of the origin of epidemic diseases and on the problem of virulence.

*Further Observations on Anti-Neutrophilic Serum.* By JOHN S. LAWRENCE and (by invitation) WILLIAM B. CHEW, Rochester, N. Y.

Guinea pigs injected with antineutrophilic serum have been treated, while the action of the antiserum was at its height, with substances which, under normal conditions, produce distinct changes in the white blood cell picture. Adrenalin subcutaneously, sodium bicarbonate solution intraperitoneally and a broth culture of *B. coli* intraperitoneally have been used. Adrenalin given to normal guinea pigs produced at 2 to 3 hours a neutrophilic leukocytosis with little or no change in the lymphocytes. Sodium bicarbonate resulted in a neutrophilic leukocytosis with lymphopenia at 6 hours; and the broth culture of *B. coli* produced at 6 hours lymphopenia without neutrophilic leukocytosis, but at 12 hours neutrophilic leukocytosis with lymphopenia, and at 24 hours neutrophilic leukocytosis with a normal lymphocyte level.

The results obtained with animals in whom the neutrophils were absent from the blood due to antiserum show that exactly the same response to these substances is obtained on the part of the lymphocytes as in normal animals. In other words, the curve for the lymphocytes, under the condition of these experiments, is independent of the response on the part of the neutrophils. Our data, also, indicate a similar response on the part of the other white blood cells.

*On the Prolonged Coagulation Time Subsequent to Anaphylactic Shock.* By HARRY EAGLE, Baltimore, Md., and (by invitation) C. G. JOHNSTON and I. S. RAVDIN, Philadelphia, Pa.

The retarded coagulation observed in rabbits and dogs immediately after anaphylactic shock is regularly asso-

ciated with the presence of increased amounts of antithrombin in the blood. The increased antithrombic activity may be as much as one hundred times the normal level.

The fibrinogen content of the plasma is unaffected; for the reasons cited in the text, there is reason to believe that even the plasmas completely non-coagulable by calcium and tissue extract nevertheless contain sufficient prothrombin to effect coagulation; and although the platelet count is usually decreased after anaphylactic shock, the amount remaining would ordinarily suffice to cause coagulation within approximately normal time limits.

The increased antithrombic activity of the blood after anaphylactic shock is apparently the primary cause of the observed retardation of coagulation.

*Scarlet Fever Immunization by Intracutaneous Injection of Scarletinal Streptococcus Toxin.* By RICHARD A. KERN, and (by invitation) JEAN CRUMP and RUDOLPH L. RODDY, Philadelphia, Pa.

In brief, the work grew out of some earlier work in our clinic with diphtheria immunization by the intracutaneous route. Subcutaneous immunization against scarlet fever has been attended by severe local and constitutional reactions in many instances. This has been particularly true among allergic individuals, so that the prophylactic treatment may be worse than the disease. The intracutaneous injection of immunizing material in one-tenth of the subcutaneous dose results in a negative Dick test, as a rule, after four or five injections. There is never a severe local reaction and we have never seen a single constitutional reaction with fever. We have given over 500 injections to over 100 patients.

*The Effect of Increased Cardiac Output in Mitral Stenosis and Aortic Insufficiency.* By EMMET B. BAY (introduced by C. Phillip Miller), Chicago, Ill.

Formulas for the calculation of the work of the appropriate part of the heart in the presence of the valvular lesions noted were tested on a model of the circulation.

In mitral stenosis:

$$\text{Left auricular work} = Vp + \frac{Vv^2}{2g} + n \frac{Vv^2}{2g},$$

where  $V$  is output per beat,  $p$  is diastolic intraventricular pressure,  $v$  is velocity,  $g$  is the force of gravity, and  $n$  is a constant for a given stenosis. Doubling the cardiac output per minute requires a relatively large increase in the work of the left auricle since it results in a greater velocity, a factor which is squared in the formula. Doubling the heart rate requires more work to overcome the stenosis than doubling the output per beat.

In aortic insufficiency:

$$\text{Left ventricular work} = (V + X)p,$$

where  $V$  is net output per beat,  $X$  is the amount re-

gurgitated and  $p$  is mean systolic intraventricular pressure.  $X$  bears a fairly direct relationship to the duration of diastole in a given insufficiency. Doubling the net output per minute requires a relatively small increase in the work of the left ventricle and may require less than with a normal output when the increased output is obtained by doubling the rate.

These calculations may give a clue to the reason for the clinical impression that patients with mitral stenosis recover more readily from myocardial insufficiency than do patients with aortic insufficiency. They do not permit an estimate of the cost of the work required.

*Venous Pressures, Cardiac Output and Blood Volume in Arteriovenous Fistula.* By C. SIDNEY BURWELL and (by invitation) J. ALLEN KENNEDY, Boston, Mass.

This report deals with the dynamics of the circulation in a young man with an arteriovenous fistula in his forearm. It deals particularly with the venous blood pressures, the total blood volume and the cardiac output: aspects of the circulation concerning which little is known relating to human subjects.

The venous pressure was increased near the fistula and at all points measured between the fistula and the heart. The venous pressure in the other limbs was not elevated.

It was necessary to test the validity in this patient of the method used for the determination of the cardiac output. To this end measurement was made of the "recirculation time," i.e., the period required for blood to travel from the pulmonary capillaries to the fistula in the forearm, pass through the abnormal connection and return by way of the veins and right heart to the lung. The period was divided into the "vein-to-lung time" and the "lung-to-vein time." The former was determined by the ether method of Hitzig, the latter was estimated by the time required for inhaled carbon monoxide to reach the vein adjacent to the fistula. Carbon monoxide hemoglobin was recognized by means of a specific light filter and a photo-electric cell. It was possible to get checks with the three sample acetylene method within the time limits thus established.

The cardiac outputs thus determined were elevated. When the fistula was closed by a blood pressure cuff on the arm, the output was within the usual limits; after operation it gradually approached the same level.

The total blood volume was determined by the method of Gregersen, Gibson and Stead,<sup>1</sup> using the blue azo dye, T-1824, and spectrophotometric analysis. The volume was elevated when the fistula was widely open, and approached the calculated normal after surgical treatment of the arteriovenous connection. The variations in cardiac output occurred at the same time and in the same direction as those in total blood volume.

<sup>1</sup> Gregersen, Magnus I., Gibson, J. J., and Stead, E. A., *Am. J. Physiol.*, 1935, 113, 54.

*Vasoconstriction as a Response to Increased Venous Pressure.*<sup>1</sup> By WILLIAM A. SODEMAN and GEORGE E. BURCH (by invitation), and ROY H. TURNER, New Orleans, La.

Volumetric changes in the finger tip were studied with the use of a sensitive syphygmoplethysmograph. Immediately proximal to a plethysmographic cup an occluding air cuff was loosely wrapped so as not to produce any constriction when deflated. The cuff was connected to a pressure reservoir and manometer so that any desired pressure might be suddenly applied and the resulting volume changes in the finger tip noted. Thirteen normal subjects, 10 patients with diastolic hypertension and one patient with acrocyanosis were studied under controlled atmospheric conditions. They were seated comfortably with the arm resting passively upon a support with the finger tip at heart level, 30 minutes being allowed to reach a steady metabolic state. Reactions were then noted for obstructing pressures varying from 5 mm. Hg to diastolic pressure and maintained for 15 to 120 seconds, an interval exceeding the time of obstruction being allowed for recovery between each observation.

In the normal subjects, for cuff pressures up to a definite critical level, varying in different individuals from 15 to 40 mm. Hg, there was a sudden increase in finger tip volume, reaching a maximum within 5 to 10 seconds after occlusion and followed by a gradual diminution in volume reaching to or below the original level within one minute (Response A). Release of cuff pressure at that time always resulted in a further diminution in volume, usually greater than the reduction occurring before pressure release. But when cuff pressures above this critical level were applied only swelling occurred, that is until pressure was released (Response B).

In the 10 patients with diastolic hypertension the responses were similar, and the levels at which the reaction changed varied from 7.5 to 60 mm. Hg. No differences were noted between the red and pale hypertension of Volhard. In a patient with acrocyanosis with arteriolar constriction and dilatation of the non-arterial vessels, a response only of Type B was observed.

The gain in volume of the finger tip immediately following application of pressure in the obstructing cuff is probably due mainly to distension of small veins, venules and capillaries, and loss of volume which followed under proper conditions is probably due to active constriction of these same vessels. The critical pressure at which the type of response changes is an index of tone of the vessels responsible for the volume change. The mechanism of the response is not known, but it is likely that it is initiated by intravascular tension.

<sup>1</sup> Aided by grants from the Josiah Macy, Jr., Foundation, the David Trautman Schwartz Research Fund and the American Medical Association.

*A Rare Form of Pararrhythmia with "Exit" Block Occurring in a Patient with Multiple Ectopic Pacemakers.* By L. N. KATZ and (by invitation) J. L. ESCHELBACHER, S. STRAUSS, S. H. ROBERTSON and H. BINSWANGER, Chicago, Ill.

This patient has shown multiple ventricular and auricular premature systoles for years, together with attacks of paroxysmal supraventricular and ventricular tachycardia and periods of bradycardia. A large number of electrocardiograms had been taken. On three occasions electrocardiograms were obtained sufficiently long for analysis, and on analysis it could be shown that each of the various ectopic pacemakers was discharging at time intervals which were multiples of a common divisor distinct for each pacemaker. It is therefore possible that they represented instances of pararrhythmia not only with interference dissociation and "entrance" block, but also with "exit" block. On one occasion a record was obtained with only one active auricular ectopic pacemaker competing with the sinus node for control of the heart. The "exit" block was marked. The sinus node was kept discharged whenever the ectopic rhythm was active, and the sinus node took control only when the "exit" block increased and prevented the ectopic pacemaker from maintaining control. We have not seen such a mechanism reported in the literature. It offers unusually convincing proof of the theory of pararrhythmia with "exit" block first postulated by Rothberger and Kaufmann.

*The Effect of Digitalis on the Anesthetized Dog.* By L. N. KATZ and (by invitation) S. ROBBARD, M. FRIEND and W. ROTTSMAN, Chicago, Ill.

In a series of acute experiments on anesthetized dogs with arterial blood pressure within normal limits, digitalis given intravenously in therapeutic doses (0.34 cat unit per kgm. as Digifoline, Ciba) elevates the arterial and portal pressures, lowers the venous pressure, and decreases the venous return flow to the heart. Our experiments indicate that these digitalis actions are due in part to peripheral vasoconstriction and in part to a constrictor action on the blood vessels of the liver. The result is a reduction in venous return and therefore in the minute volume output of the heart. These effects persist but are less in degree when the portal blood is shunted directly to the vena cava without passing through the liver.

In anesthetized dogs with arterial blood pressures at shock level, results are the same as above except that the venous return flow to the heart is increased. These effects also persist when the portal blood is diverted from the liver. The major effect of digitalis at low blood pressure appears to be the result of vasoconstriction improving the deficient coronary circulation by elevating the arterial blood pressure. In this way the heart action is benefited and the minute volume output of the heart is increased. The reduction in venous pressure load on the right heart would enhance this action.

While the experiments reported by us have not ruled out an action of digitalis directly on the heart muscle, they show that such a possible action need not be invoked to explain our observations.

*The Electrocardiogram in Air and Fat Embolism.* By THOMAS M. DURANT (introduced by Charles L. Brown), Philadelphia, Pa.

A series of experiments was carried out, using the dog as the experimental animal, to determine the type and origin of electrocardiographic changes associated with air and fat embolism originating in the systemic venous circulation. The electrocardiographic changes obtained were of an extreme type and were remarkable for their constancy in all experiments regardless of whether air or fat was the embolic substance. These changes consisted in very marked lowering of the S-T segment in Leads II and III, without appreciable change in Lead I, and not associated with alteration of the QRS complex, corresponding with changes often seen in infarction of the myocardium. That these changes were not, however, due to coronary artery embolism was proven by pathological study. They occurred in association with a marked, sudden rise in venous pressure, and, in animals with the thorax open, appeared synchronously with the onset of right ventricular dilatation. It is concluded from the evidence made available by these experiments that the electrocardiographic changes observed were those of acute right heart dilatation secondary to obstruction of the pulmonary circulation by the embolic substance. The similarity to S-T changes observed in cases of human pulmonary embolism is discussed.

*Factors Which Affect the Prognosis of Bundle Branch Block.* By A. CARLTON ERNSTENE, Cleveland, Ohio.

That bundle branch block usually is associated with serious organic heart disease is a matter of common knowledge. It is not so generally recognized, however, that the prognosis in patients with this type of electrocardiographic abnormality is determined by the cardiovascular symptoms and signs, which are so often present, rather than by the bundle branch block itself. In the absence of congestive failure or of the anginal syndrome, bundle branch block is not incompatible with several years of life.

In a series of 66 consecutive cases of bundle branch block, there were 7 patients in whom the abnormality is known to have been present for two to ten years and in whom significant symptoms referable to the heart have not developed. Six other patients have had bundle branch block for at least one year and have remained free from symptoms. The case records of these patients have been analyzed, and a comparison has been made with the clinical findings in the patients who have died or who are still living but are experiencing cardiovascular symptoms.

*Systolic Gallop Rhythm.* By FRANKLIN D. JOHNSTON, Ann Arbor, Mich.

Twenty-one patients displaying on auscultation extra sounds in cardiac systole have been studied by the commonly used clinical methods and by means of sound tracings taken simultaneously with the electrocardiogram.

The clinical data obtained confirms the opinions of

previous investigators that, first, systolic clicks usually occur in individuals who show no evidence of organic heart disease and that they therefore have no unfavorable prognostic significance, and second, that their sole clinical importance lies in the possibility that they may be mistaken for the more common diastolic gallop sounds.

Measurements made from a fixed point of the accompanying electrocardiogram show that, with few exceptions, the position of the extra sound from cycle to cycle is much more variable than is the onset of the second heart sound.

Although the exact cause for systolic clicks remains obscure we believe that their clinical characteristics and the results of the measurements indicate that while they are intimately associated with movements of the heart within the chest they are not intracardiac in the sense that they are due to pressure changes within the cavities of the heart or the aorta.

*The Sedimentation Rate in Angina Pectoris and Coronary Thrombosis.* By JOSEPH E. F. RISEMAN and MORTON G. BROWN (introduced by Samuel L. Gargill), Boston, Mass.

The corrected sedimentation index was studied in thirty-seven cases of coronary thrombosis, fifty-five patients with angina pectoris, and twenty-one apparently normal persons of similar age.

It was evident that individuals between the ages of 45 and 70, without any evidence of disease, may have a corrected sedimentation index slightly higher (up to 0.70) than the accepted normal for young adults.

Over half of the patients with angina pectoris had a moderate elevation of the sedimentation index (0.73 to 1.38). There is reason to believe that attacks of angina pectoris occasionally result in myocardial damage.

Two-thirds of the cases of coronary thrombosis had rates considerably greater than that seen in any patients with angina pectoris; in the remaining third, the values were above 0.1 mm. The fastest rates were observed between the fourth and twelfth days after the onset of symptoms of coronary occlusion. Measurement of the sedimentation rate is of value in differentiating between angina pectoris and coronary occlusion. The sedimentation rate is of little or no aid in predicting the immediate outcome of the acute attack. The mortality of patients discharged from the hospital with fast rates, however, was twice as great during the first year after discharge as that of patients discharged with low or normal rates. Since the sedimentation rate signifies tissue damage, it is advisable to continue bed rest until the rate either returns to normal or shows no further progression towards normal.

*A Clinical Study of the Action of Commonly Used Drugs on the Heart and Circulation.* By C. J. GAMELE, ISAAC STARR and (by invitation) A. MARCOLIES, J. S. DONAL, JR., N. JOSEPH and E. EAGLE, Philadelphia, Pa.

The subjects consisted of about 100 patients suffering chiefly from cardiac or circulatory disease, but not from

congestive heart failure. As far as possible we studied the action of drugs given under the conditions in which physicians are accustomed to employ them. The number of patients receiving a single drug varied from 4 to 14.

The drugs investigated included digitalis, epinephrine, ephedrine, caffeine, theophylline, carbaminoyleholine, sodium nitrite, nitroglycerine, pitressin, quinidine, morphine, and strychnine.

The study consisted of a group of estimations made before, during, and sometimes after, the drugs' action. This group consisted of duplicate determinations of cardiac output and metabolic rate, and repeated estimations of pulse rate, blood pressure, respiratory rate and volume. Orthodiagrams and electrocardiograms were secured also.

A statistical analysis of the data affords a basis for describing the action to be expected after the administration of these drugs in clinical conditions. Almost without exception the results support the conceptions of drug action derived from animal experiments.

*The Effect of Epinephrin in Circulatory Collapse.* By ROBERT W. WILKINS (by invitation) and SOMA WEISS, Boston, Mass.

Nitrite collapse induced in normal subjects was used to test the therapeutic efficacy of epinephrin. Epinephrin produces elevation of the arterial pressure with essentially unchanged venous pressure both in the horizontal and in the upright position. As indicated by the behavior of the vessels of the hand studied by plethysmographic methods, the arteries and arterioles become sharply constricted and the blood flow decreases. The venous tone, however, remains unaltered or only moderately increased. When epinephrin, in doses which produce therapeutic or slightly toxic effects, is given soon after the administration of nitrites but before the appearance of symptoms, all the manifestations of collapse and syncope develop just as soon as, if not sooner than in the controls, in spite of the fact that the fall of arterial pressure associated with nitrite collapse may be partially or completely prevented. In the presence of collapse, epinephrin augments the already existing arterial and arteriolar constriction with decreased blood flow but fails to produce significant constriction of the capillary and venous reservoirs. The venous return of blood to the right side of the heart is not enhanced. These observations indicate that the level of the arterial pressure is not necessarily a criterion of circulatory collapse. It is concluded that in spite of its arteriopressor effect, epinephrin may be harmful in certain types of collapse as it may further enhance tissue anoxia.

*Changes in Blood Volume in Congestive Heart Failure.*

By WILLIAM A. EVANS, JR., and JOHN G. GIBSON, 2d (introduced by Henry A. Christian), Boston, Mass.

Changes in plasma and total blood volume occurring in chronic heart disease during the transition from compensation to decompensation, and during clinical recovery

from congestive heart failure were studied by means of the dye method of determining the blood volume described by Gibson and Evans (J. Clin. Invest., 1937, 16, 301).

The change from the compensated to the decompensated state is characterized by a progressive increase in the volume of plasma and red blood cells. This increase is shared to a less extent by the plasma than by the red cells resulting in a slight concentration of the blood, as evidenced by an increase above normal levels in hematocrits. The degree of elevation of blood volume above the average normal level, as determined in 90 individuals, parallels the degree of elevation of venous pressure and increase in circulation time above average normal values.

During clinical recovery from congestive heart failure there is a diminution in both plasma and red cell volume, the decrease in plasma volume being at first more rapid than that of the red cell volume, resulting in still further concentration of the blood. With continued improvement the proportion of red cells to plasma tends to return to normal levels. The diminution in total volume is commensurate with the degree of clinical improvement.

An increase in blood volume over levels obtaining during failure was not observed in any case studied during recovery from chronic congestive heart failure. Relapses to more severe degrees of circulatory failure are accompanied by maintained elevation of or further increase in blood volume.

*The Influence of Theophylline upon the Absorption of Mercurial Diuretics from the Site of Injection.* By ARTHUR C. DEGRAFF and (by invitation) ROBERT A. LEHMAN and ROBERT C. BATTERMAN, New York, N. Y.

It has previously been shown that a mercurial diuretic containing theophylline (mercupurin) is capable of producing a greater diuresis than a simple mercurial (salyrgan). Furthermore, tissue destruction noted at the site of injection with a mercurial diuretic was for the most part prevented by the presence of theophylline in sufficient concentration in the injected solution (DeGraff, Nadler, and Batterman).

In the light of these results it was thought to be of interest to determine quantitatively the rates of absorption of mercurials from muscle with and without the addition of theophylline. Experimentally, this was carried out by giving a series of rabbits intramuscular injections of mercupurin and salyrgan. The animals were killed at definite time intervals and the amount of mercury remaining at the site of injection determined by chemical analysis of the muscle used. By this procedure we have come to the following conclusions: (1) that a mercurial diuretic containing theophyllin is completely absorbed one hour after intramuscular injection whereas a mercurial diuretic without theophyllin is only 25 per cent absorbed after the same interval; (2) that a mercurial diuretic without theophyllin is still only 80 per cent absorbed after 48 hours; and (3) that this marked difference in the rates of absorption of the two drugs is entirely due to theophyllin.

*Prostigmin in the Diagnosis and Treatment of Myasthenia Gravis.* By GEO. D. GAMMON and (by invitation) E. A. SCHEIE, Philadelphia, Pa.

Relief of myasthenia gravis by eserine and prostigmin suggests the mechanism involved is a failure of the motor nerve impulse to activate the muscle fiber; deficiency of neurohumoral transmission may be involved. The possibility of utilizing the response to prostigmin as a diagnostic test of myasthenia is not widely appreciated. Schwab and Viets have shown cases of upper and lower motor neurone paralysis are not relieved by the drug. In this study we found that various muscular diseases likewise failed to respond. The cases include progressive muscular dystrophy, myotonic atrophy, family periodic paralysis, and amyotonia congenita. Thus the only condition so far examined which is relieved by prostigmin is myasthenia gravis; hence the drug can be used to diagnose the disease. In the treatment of myasthenia, symptomatic relief may be obtained; it appears likely the natural course of the disease is uninfluenced.

*The Interpretation of Pathological Joint Effusions.* By H. C. COGGESHALL, MARIAN ROPES and ELSIE ROSSMEISL (by invitation), and WALTER BAUER, Boston, Mass.

The cytological, physical and chemical characteristics of normal bovine and human synovial fluid have been established. Normal human synovial fluid is more viscous and the concentration of serum proteins and mucin are greater. Both fluids are relatively acellular. The predominant cells are monocytes.

Having defined normal synovial fluid, we are in a better position to interpret the variations that we have encountered in pathological fluids obtained from patients with various types of joint disease.

Edema from any cause results in a lowering of the total cell count, mucin and protein. Most of the protein is albumin.

The total cell count is increased in hypertrophic arthritis. Both total and polymorphonuclear cell counts are increased in traumatic effusions. The total cell and polymorphonuclear cell counts increase progressively with increasing inflammation of the synovial membrane.

There is no absolute relationship between sugar content and cell counts. Some of the lowest sugar values have been observed in rheumatoid arthritis. The total protein and globulin content increases with increasing inflammation of the synovial membrane, the highest values being encountered in long standing effusions of rheumatoid arthritis. Mucin increases are seen only in non-inflammatory joint diseases.

These and similar data from rheumatoid arthritis with regularly recurring knee joint effusions show that the cytological and chemical variations depend on the severity and duration of the inflammation of the synovial membrane as well as on the etiology. If these facts and the clinical history are taken into account, ex-

aminations of the synovial fluid are of diagnostic and prognostic significance.

*Calcium and Phosphorus Metabolism Studies in Rheumatoid and Degenerative Arthritis.* By MARIAN ROPES (by invitation), CHARLES L. SHORT and ELSIE ROSSMEISL (by invitation), and WALTER BAUER, Boston, Mass.

The marked decalcification observed in rheumatoid arthritis and the osteophyte formation seen in hypertrophic arthritis suggest the possibility of an altered calcium and phosphorus metabolism in these two diseases.

The fasting serum calcium, phosphorus and phosphatase values in such individuals were found to be within normal limits. Therefore, complete calcium, phosphorus and nitrogen metabolism studies were carried out on eight rheumatoid and three hypertrophic arthritics. When studied on a low calcium intake, the observed negative calcium balances in both groups were essentially the same as in the controls. The four patients with rheumatoid arthritis exhibiting the most marked decalcification had the highest negative calcium balances. That marked restriction of activity is an adequate explanation for the observed increased calcium excretion is suggested by the fact that immobilization of a normal individual in a cast will result in an increased calcium excretion of 0.95 gram per three-day period. Four rheumatoid arthritic patients studied on a high calcium diet showed no abnormalities. The actual phosphorus balances were in all cases in close agreement with the theoretical phosphorus balances.

These results indicate that an altered calcium and phosphorus metabolism is not a primary feature of either rheumatoid or hypertrophic arthritis.

*The Mechanism of Human Diabetes Insipidus.* By HENRY L. SCHMITZ, Chicago, Ill.

The nature of the disturbance in water metabolism in diabetes insipidus has been studied in four patients with this disease. The following observations were made. The withdrawal of water for periods of four to six hours had no significant effect upon the urine output although the patients complained of thirst. When water was then allowed ad libitum the output was influenced but little, and the enormously excessive intake approximately made up the deficit created during the period of abstinence. Analysis of renal function by the creatinine clearance method of Rehberg indicated that the polyuria was due to a deficient reabsorption of water in the renal tubules. Glomerular filtration was within normal limits. The administration of pituitrin decreased the urine volume by increasing tubular reabsorption. As the effect of pituitrin wore off tubular reabsorption decreased and urine volume increased. Increase in the fluid intake lagged behind the return of the polyuria.

These results are interpreted as meaning that the essential defect in diabetes insipidus is renal, specifically a failure in tubular reabsorption of water and that pituitrin corrects this defect.



*Effect on Tumor Growth of Treatment with Colchicine and X-rays.* By AUSTIN M. BRUES and BEULA B. MARBLE (introduced by Joseph C. Aub), Boston, Mass.

The alkaloid colchicine is a specific poison for cell division, blocking mitosis in the visible metaphase stage. It is also known to have a specific metabolic effect on tissues, consisting in marked reduction in vitamin C content. The present experiments have been performed on rats and mice bearing experimental tumors, and indicate that the administration of repeated large doses of colchicine retards the growth of these tumors very markedly, although regression of tumors rarely occurs. This seems to be due in part to the effect of the drug on cell division, and in part to vascular injury such as occurs after administration of bacterial filtrates. Retardation occurs only with toxic or near-toxic doses, and many of these animals died of colchicine poisoning. A series of animals receiving smaller doses, in amounts that could safely be administered daily, showed no tendency to retardation of growth and no lowering of tissue or tumor ascorbic acid, although some arrest of mitosis was seen. The smaller doses of colchicine, when combined with x-ray treatment (300 r daily for ten days) caused no further regression of tumors than did x-rays alone.

*Insensible Water Loss in Disease.* By ALEXANDER W. WINKLER (introduced by John P. Peters), New Haven, Conn.

The daily insensible loss of weight over considerable periods of time was determined in a group of some twenty patients presenting various metabolic disturbances. In 6 edematous nephritic subjects the insensible loss did not vary significantly with urine flow, fluid intake, or change in body water and salt content during diuresis. In both edematous and non-edematous subjects the average insensible loss was definitely correlated with the basal metabolism. The daily insensible loss varied more in individual subjects than did the basal metabolism. In certain cases of renal and vascular disease the average insensible loss over prolonged periods was found to be related to the total metabolism by the following approximate equation:

$$\text{Calories burned} = 2.0 \times \text{I. L. (grams)}.$$

Evidence, however, is presented suggesting that the factor may be greater than 2.0 in certain of the edematous and less than 2.0 in the hyperthyroid cases. In one case of recovered acute nephritis with malnutrition, there was a definite discrepancy between the total metabolism calculated from the insensible loss by this equation, and the insensible loss as estimated from indirect calorimetry. It is concluded that while insensible loss is closely dependent on total metabolism in disease as well as in health, under some circumstances the quantitative relationship may be different.

*The Metabolism of Phosphorus in Periodic Family Paralysis.* By A. T. MILHORAT (introduced by Eugene F. DuBois), New York, N. Y.

The metabolism of calcium, phosphorus, and magne-

sium was studied in a patient with periodic family paralysis. The patient was a 15 year old male who had periodic attacks of paralysis involving most of the voluntary muscles, lasting for about 24 hours. Following the occurrence of a few of these attacks, the urinary and fecal output of calcium, phosphorus, and magnesium was determined for 8 periods of 6 days each during which carefully weighed diets were given. The mineral content of the diet was kept constant from day to day during each period, but varied for the different periods. The calcium balance was normal. The patient was in balance on a diet containing 1.0 gram of calcium daily. On a daily intake of 2.0 grams of calcium, 0.550 gram were retained daily; on a daily intake of 0.100 gram there was a negative balance of 0.260 gram. Likewise, the output of magnesium was normal. The amounts of magnesium eliminated daily were similar to those in the diet on intakes varying from 0.162 gram to 0.306 gram. On the other hand, there was a negative balance of phosphorus during all the periods of investigation. During the periods when 1.6 grams of phosphorus was contained in the daily diet, the average daily negative balance was 0.362 gram. On an intake of 2.0 grams the average daily negative balance was 0.147 gram and when 0.60 gram of phosphorus was given daily the amounts eliminated exceeded the intake by 0.358 gram. By the use of similar methods and diets the mineral balance of 2 patients with progressive muscular dystrophy and 2 patients with myotonia atrophica was determined. In these 4 patients without periodic family paralysis the metabolism of calcium, phosphorus, and magnesium was normal.

The data suggest that in periodic family paralysis there is a disturbance in the metabolism of phosphorus without any involvement of the metabolism of calcium or magnesium. These observations were made after the occurrence of a few attacks of paralysis. Whether a similar loss of phosphorus precedes an attack of paralysis or whether phosphorus is retained at that time has not yet been determined.

*Further Experiments on Experimental Hyposthenuria.* By PAUL DUMKE (by invitation) and J. M. HAYMAN, JR., Cleveland, Ohio.

This is a continuation of experiments reported last spring by Shumway and Hayman. At that time, it was shown that dogs subjected to subtotal nephrectomy excreted an increased volume of dilute urine, but could be made to put out a concentrated urine under certain experimental conditions (intravenous sodium sulphate, increased plasma colloids, low blood pressure). Dogs poisoned by uranium, on the other hand, could not be made to excrete a concentrated urine under any circumstances.

In the present experiments, the effects of ureteral obstruction have been studied. Dogs subjected to adequate ureteral obstruction excrete a dilute urine, and cannot be made to concentrate by withholding food and water, nor by any methods tried. The daily urine volume is usually increased. Creatinine and urea clearances are markedly reduced. Blood pressure is not significantly elevated.

These changes in renal function are apparent as early as five days after obstructing the ureters. Histologically, such animals show tubular degeneration. This damage, however, is reversible, for if the ureteral obstruction is removed the animal recovers its ability to excrete a urine of high gravity.

*Successful Management of Addison's Disease with Adrenal Cortex Extract.* By W. O. THOMPSON and (by invitation) P. K. THOMPSON, S. G. TAYLOR, III, and W. S. HOFFMAN, Chicago, Ill.

We have been able to rehabilitate patients with typical Addison's disease and maintain them in good health for long periods by the administration of large doses of an adrenal cortex extract (10 to 20 cc. daily) without any other form of therapy. When typical symptoms of adrenal insufficiency develop during the administration of an inadequate dose of the extract, or following its omission, the patient may be revived by large doses of the extract alone, provided the insufficiency has not been allowed to develop too far. When a crisis is well marked the administration of sodium salts must be added to the treatment until nausea and vomiting disappear. While patients may be maintained for periods of several months by the administration of sodium salts alone or by combining this form of therapy with small doses of extract, the most desirable clinical condition is not produced unless an adequate dose of extract is given, and then supplementing with sodium salts is unnecessary. If the dose of extract is adequate, the potassium content of the diet may be ignored. While changes in the composition of the blood are important in diagnosis and as an index of the efficacy of treatment, the data show that, early in a crisis, there may be some discrepancy between the concentration of various substances in the blood and the clinical condition of the patient. When the crisis is well marked, the blood findings usually coincide with the clinical condition.

*The Role in Growth and Development of Certain Potent Chemical Agents Found in Thymus Extract.* By L. G. ROWNTREE, and (by invitation) ARTHUR STEINBERG, N. H. EINHORN, N. K. SCHAFER and WILLIAM ZIEGLER, Philadelphia, Pa.

With freshly prepared solutions of glutathione, ascorbic acid and cysteine administered to parent rats, we have produced definite acceleration in the rate of growth and development of the offspring, in the second generation. These may be used singly or in combination. They are now being tried by way of substitution therapy in the retardation in growth and development incident to thyrectomy, through successive generations of rats. Ergothioneine, another iodine-reducing agent, is not present in the thymus extract. The effects of glutamic acid and glycine are under investigation.

The possibility of obtaining thymus effects from a synthetic extract containing glutathione, ascorbic acid and cysteine is being tried at present.

*The Treatment of Diabetes Mellitus with Protamine Zinc Insulin.* By S. SOSKIN, and (by invitation) R. LEVINE and M. D. ALLWEISS, Chicago, Ill.

There can be no doubt as to the greater efficacy and convenience of protamine zinc insulin, in the treatment of diabetes mellitus, as evidenced by a number of recent clinical reports. However, further experience has tempered our initial enthusiasm with certain practical considerations. Treatment with protamine zinc insulin was instituted under carefully controlled conditions in the Max Pam Metabolism Unit of the Michael Reese Hospital. After several weeks of hospital observation, these same cases were followed for many months in the outpatient dispensary. Our observations on these and other patients have led us to the following conclusions: (a) A single dose of protamine insulin may show some effect for as long as 48 hours in a fasting individual, but its clinically significant period of activity under the ordinary conditions of feeding is probably 16 to 20 hours. (b) Diabetes of the adult type can usually be well controlled with one dose of protamine insulin per day. (c) Diabetes of the juvenile type can be well controlled but usually requires two doses of protamine insulin per day. (d) Occasional severe juvenile diabetics cannot be well controlled throughout the 24 hours of the day by either protamine or ordinary insulin. (e) Because of its lesser effect on alimentary hyperglycemia, protamine insulin may be less effective than ordinary insulin in the treatment of the uncoöperative or unintelligent patient.

*Indirect Calorimetric Study of the Oxidation of Glucose in Controlled and Uncontrolled Diabetic Men.* By J. M. SHELTON (by invitation) and L. H. NEWBURGH, Ann Arbor, Mich.

The study consisted of a continuous four hour measurement by means of a respiration chamber of the amount of carbohydrate oxidized by normal men and by diabetics when they were controlled and uncontrolled. Standard, open circuit, indirect calorimetry was employed. All subjects were first studied while subsisting on a low carbohydrate, high fat type of diet. Insulin was not employed. The diets for all subjects were adjusted so that the blood sugars of the diabetics remained within the normal range. The energy of the diet was approximately maintenance. Subsequently, the oxidation of glucose by the same diabetics was studied while they were constantly hyperglycemic and glycosuric, due to the addition of sufficient carbohydrate to the diet (calories were kept constant by an isocaloric reduction of fat). The controls received the same diet. A typical example follows:

	Carbohydrate of preparatory diet	Glucose at beginning of indirect calorimetry	Glucose oxidized
	grams	grams	grams
Normals.....	50	50	22
Diabetic controlled.....	50	50	18
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Diabetic uncontrolled...	130	100	20



*Effect on Tumor Growth of Treatment with Colchicine and X-rays.* By AUSTIN M. BRUES and BEULA B. MARBLE (introduced by Joseph C. Aub), Boston, Mass.

The alkaloid colchicine is a specific poison for cell division, blocking mitosis in the visible metaphase stage. It is also known to have a specific metabolic effect on tissues, consisting in marked reduction in vitamin C content. The present experiments have been performed on rats and mice bearing experimental tumors, and indicate that the administration of repeated large doses of colchicine retards the growth of these tumors very markedly, although regression of tumors rarely occurs. This seems to be due in part to the effect of the drug on cell division, and in part to vascular injury such as occurs after administration of bacterial filtrates. Retardation occurs only with toxic or near-toxic doses, and many of these animals died of colchicine poisoning. A series of animals receiving smaller doses, in amounts that could safely be administered daily, showed no tendency to retardation of growth and no lowering of tissue or tumor ascorbic acid, although some arrest of mitosis was seen. The smaller doses of colchicine, when combined with x-ray treatment (300 r daily for ten days) caused no further regression of tumors than did x-rays alone.

*Insensible Water Loss in Disease.* By ALEXANDER W. WINKLER (introduced by John P. Peters), New Haven, Conn.

The daily insensible loss of weight over considerable periods of time was determined in a group of some twenty patients presenting various metabolic disturbances. In 6 edematous nephritic subjects the insensible loss did not vary significantly with urine flow, fluid intake, or change in body water and salt content during diuresis. In both edematous and non-edematous subjects the average insensible loss was definitely correlated with the basal metabolism. The daily insensible loss varied more in individual subjects than did the basal metabolism. In certain cases of renal and vascular disease the average insensible loss over prolonged periods was found to be related to the total metabolism by the following approximate equation:

$$\text{Calories burned} = 2.0 \times \text{I. L. (grams)}.$$

Evidence, however, is presented suggesting that the factor may be greater than 2.0 in certain of the edematous and less than 2.0 in the hyperthyroid cases. In one case of recovered acute nephritis with malnutrition, there was a definite discrepancy between the total metabolism calculated from the insensible loss by this equation, and the insensible loss as estimated from indirect calorimetry. It is concluded that while insensible loss is closely dependent on total metabolism in disease as well as in health, under some circumstances the quantitative relationship may be different.

*The Metabolism of Phosphorus in Periodic Family Paralysis.* By A. T. MILHORAT (introduced by Eugene F. DuBois), New York, N. Y.

The metabolism of calcium, phosphorus, and magne-

sium was studied in a patient with periodic family paralysis. The patient was a 15 year old male who had periodic attacks of paralysis involving most of the voluntary muscles, lasting for about 24 hours. Following the occurrence of a few of these attacks, the urinary and fecal output of calcium, phosphorus, and magnesium was determined for 8 periods of 6 days each during which carefully weighed diets were given. The mineral content of the diet was kept constant from day to day during each period, but varied for the different periods. The calcium balance was normal. The patient was in balance on a diet containing 1.0 gram of calcium daily. On a daily intake of 2.0 grams of calcium, 0.550 gram were retained daily; on a daily intake of 0.100 gram there was a negative balance of 0.260 gram. Likewise, the output of magnesium was normal. The amounts of magnesium eliminated daily were similar to those in the diet on intakes varying from 0.162 gram to 0.306 gram. On the other hand, there was a negative balance of phosphorus during all the periods of investigation. During the periods when 1.6 grams of phosphorus was contained in the daily diet, the average daily negative balance was 0.362 gram. On an intake of 2.0 grams the average daily negative balance was 0.147 gram and when 0.60 gram of phosphorus was given daily the amounts eliminated exceeded the intake by 0.358 gram. By the use of similar methods and diets the mineral balance of 2 patients with progressive muscular dystrophy and 2 patients with myotonia atrophica was determined. In these 4 patients without periodic family paralysis the metabolism of calcium, phosphorus, and magnesium was normal.

The data suggest that in periodic family paralysis there is a disturbance in the metabolism of phosphorus without any involvement of the metabolism of calcium or magnesium. These observations were made after the occurrence of a few attacks of paralysis. Whether a similar loss of phosphorus precedes an attack of paralysis or whether phosphorus is retained at that time has not yet been determined.

*Further Experiments on Experimental Hyposthenuria.*

By PAUL DUMKE (by invitation) and J. M. HAYMAN, JR., Cleveland, Ohio.

This is a continuation of experiments reported last spring by Shumway and Hayman. At that time, it was shown that dogs subjected to subtotal nephrectomy excreted an increased volume of dilute urine, but could be made to put out a concentrated urine under certain experimental conditions (intravenous sodium sulphate, increased plasma colloids, low blood pressure). Dogs poisoned by uranium, on the other hand, could not be made to excrete a concentrated urine under any circumstances.

In the present experiments, the effects of ureteral obstruction have been studied. Dogs subjected to adequate ureteral obstruction excrete a dilute urine, and cannot be made to concentrate by withholding food and water, nor by any methods tried. The daily urine volume is usually increased. Creatinine and urea clearances are markedly reduced. Blood pressure is not significantly elevated.

These changes in renal function are apparent as early as five days after obstructing the ureters. Histologically, such animals show tubular degeneration. This damage, however, is reversible, for if the ureteral obstruction is removed the animal recovers its ability to excrete a urine of high gravity.

*Successful Management of Addison's Disease with Adrenal Cortex Extract.* By W. O. THOMPSON and (by invitation) P. K. THOMPSON, S. G. TAYLOR, III, and W. S. HOFFMAN, Chicago, Ill.

We have been able to rehabilitate patients with typical Addison's disease and maintain them in good health for long periods by the administration of large doses of an adrenal cortex extract (10 to 20 cc. daily) without any other form of therapy. When typical symptoms of adrenal insufficiency develop during the administration of an inadequate dose of the extract, or following its omission, the patient may be revived by large doses of the extract alone, provided the insufficiency has not been allowed to develop too far. When a crisis is well marked the administration of sodium salts must be added to the treatment until nausea and vomiting disappear. While patients may be maintained for periods of several months by the administration of sodium salts alone or by combining this form of therapy with small doses of extract, the most desirable clinical condition is not produced unless an adequate dose of extract is given, and then supplementing with sodium salts is unnecessary. If the dose of extract is adequate, the potassium content of the diet may be ignored. While changes in the composition of the blood are important in diagnosis and as an index of the efficacy of treatment, the data show that, early in a crisis, there may be some discrepancy between the concentration of various substances in the blood and the clinical condition of the patient. When the crisis is well marked, the blood findings usually coincide with the clinical condition.

*The Role in Growth and Development of Certain Potent Chemical Agents Found in Thymus Extract.* By L. G. ROWNTREE, and (by invitation) ARTHUR STEINBERG, N. H. EINHORN, N. K. SCHAEFFER and WILLIAM ZIEGLER, Philadelphia, Pa.

With freshly prepared solutions of glutathione, ascorbic acid and cysteine administered to parent rats, we have produced definite acceleration in the rate of growth and development of the offspring, in the second generation. These may be used singly or in combination. They are now being tried by way of substitution therapy in the retardation in growth and development incident to thymectomy, through successive generations of rats. Ergothioneine, another iodine-reducing agent, is not present in the thymus extract. The effects of glutamic acid and glycine are under investigation.

The possibility of obtaining thymus effects from a synthetic extract containing glutathione, ascorbic acid and cysteine is being tried at present.

*The Treatment of Diabetes Mellitus with Protamine Zinc Insulin.* By S. SOSKIN, and (by invitation) R. LEVINE and M. D. ALLWEISS, Chicago, Ill.

There can be no doubt as to the greater efficacy and convenience of protamine zinc insulin, in the treatment of diabetes mellitus, as evidenced by a number of recent clinical reports. However, further experience has tempered our initial enthusiasm with certain practical considerations. Treatment with protamine zinc insulin was instituted under carefully controlled conditions in the Max Pam Metabolism Unit of the Michael Reese Hospital. After several weeks of hospital observation, these same cases were followed for many months in the outpatient dispensary. Our observations on these and other patients have led us to the following conclusions: (a) A single dose of protamine insulin may show some effect for as long as 48 hours in a fasting individual, but its clinically significant period of activity under the ordinary conditions of feeding is probably 16 to 20 hours. (b) Diabetes of the adult type can usually be well controlled with one dose of protamine insulin per day. (c) Diabetes of the juvenile type can be well controlled but usually requires two doses of protamine insulin per day. (d) Occasional severe juvenile diabetics cannot be well controlled throughout the 24 hours of the day by either protamine or ordinary insulin. (e) Because of its lesser effect on alimentary hyperglycemia, protamine insulin may be less effective than ordinary insulin in the treatment of the uncoöperative or unintelligent patient.

*Indirect Calorimetric Study of the Oxidation of Glucose in Controlled and Uncontrolled Diabetic Men.* By J. M. SHELDON (by invitation) and L. H. NEWBURGH, Ann Arbor, Mich.

The study consisted of a continuous four hour measurement by means of a respiration chamber of the amount of carbohydrate oxidized by normal men and by diabetics when they were controlled and uncontrolled. Standard, open circuit, indirect calorimetry was employed. All subjects were first studied while subsisting on a low carbohydrate, high fat type of diet. Insulin was not employed. The diets for all subjects were adjusted so that the blood sugars of the diabetics remained within the normal range. The energy of the diet was approximately maintenance. Subsequently, the oxidation of glucose by the same diabetics was studied while they were constantly hyperglycemic and glycosuric, due to the addition of sufficient carbohydrate to the diet (calories were kept constant by an isocaloric reduction of fat). The controls received the same diet. A typical example follows:

	Carbohydrate of preparatory diet	Glucose at beginning of indirect calorimetry	Glucose oxidized
	grams	grams	grams
Normals.....	50	50	22
Diabetic controlled.....	50	50	18
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solution intraperitoneally, and (3) repeated daily doses of liver extract intramuscularly for one week. In each instance the amount injected was arbitrarily chosen: 0.5 cc. per 100 grams of body weight. The normal rats receiving liver extract and the anemic rats which were given saline solution failed to show reticulocyte reactions. However, significant responses were observed in the anemic animals which received either one injection or repeated daily injections of fresh commercial liver extract. An attempt was made to control these experiments by eliminating other factors known to cause reticulocyte reactions.

*The Etiology of Idiopathic Hypochromic Anemia.* By W. M. FOWLER and (by invitation) ADELAIDE P. BARER, Iowa City, Iowa.

During the course of studies of iron metabolism on patients with hypochromic anemia certain observations pertaining to the etiology of "idiopathic hypochromic anemia" were made.

Determinations of iron balance show that patients with achlorhydria retain less iron from a normal dietary iron intake than do patients with a normal gastric acidity.

Although the patients with idiopathic hypochromic anemia denied excessive menstrual blood loss, it was found that the average loss for these individuals was considerably larger than the average for 100 normal women. It was also found that the amount of iron which these patients retained from a normal dietary intake was not sufficient to replace that lost by menstruation.

A daily iron intake of approximately 12 mgm. is apparently necessary to maintain a positive balance.

The patients with idiopathic hypochromic anemia retained as much iron and regenerated hemoglobin as rapidly while receiving medicinal iron as did patients with chronic hemorrhagic anemia, so that no evidence of faulty iron metabolism was apparent.

*Blood Volume Changes in Pernicious Anemia.* By J. G. GIBSON, 2d (by invitation) and WILLIAM P. MURPHY, Boston, Mass.

Changes in plasma and red blood cell volume occurring in patients with pernicious anemia treated by parenteral liver extract were studied by the method described by Gibson and Evans (J. Clin. Invest., 1937, 16, 301).

In severe anemia the total blood volume is reduced, averaging 17.5 per cent below normal in six patients with red cell counts around 1,500,000. The diminution in red cell volume is offset to some extent by an increase in the plasma volume. The individual cell volume is high.

With clinical improvement there is a progressive increase in the total blood volume, the plasma volume tending to diminish as the red cell volume rises. With complete clinical recovery the red cell and total blood volume returns to normal limits. Changes in individual cell volume vary with individuals but the general trend is toward smaller cells as red cell volume rises.

During recovery, the relationship between changes in plasma and red cell volume is such that the degree of re-

turn in red cell volume to normal values is closely reflected by the degree of return to normal values of red cell counts, hemoglobin determinations and hematocrit values.

Certain evidence has been obtained which indicates that the response, in terms of percentage return to normal in red cell volume is slower following multiple small doses of liver extract than that which follows single large doses, even though a satisfactory reticulocyte response occurs in both instances.

*The Maturation of Transfused Reticulocytes in the Rat.*

By A. J. CRESKOFF (by invitation) and THOMAS FITZHUGH, Jr., Philadelphia, Pa.

Our previous study<sup>1</sup> of the reticulocytosis of fetal and nursing rats indicated the probability of peripheral reticulocyte maturation.

In the present experiments litters of young rats with high reticulocyte counts were used to supply donor-blood to a recipient group of adult rats with low reticulocyte counts. Just before transfusion, recipients were bled an amount equal to the volume to be transfused (average 5 cc.). In another group, anemic adult animals, with high reticulocyte counts induced by repeated bleedings, were used as donors. In both groups the donor blood was concentrated to an 8,000,000 erythrocyte count before transfusion. Control bleeding and transfusion experiments were made with normal adult animals.

In all of our experiments the transfused reticulocytes disappeared progressively over a period of 48 to 96 hours, at the end of which time the recipient's reticulocyte count had declined to pre-transfusion level without any concomitant change in the recipient's elevated post-transfusion erythrocyte counts. This we believe indicates peripheral reticulocyte maturation and suggests that the youngest reticulocytes (rat) require almost 4 days to "mature" in the peripheral blood. Our data suggest also that transfused erythrocytes survive for 5 to 8 days. Wistar-strain albino rats have no demonstrable intra-group hemagglutinins.

*The Questionable Relationship of Staphylococcus Infection to Leukemia.* By FRANKLIN R. MILLER, Cleveland, Ohio.

Infection has been considered frequently as an etiological agent in the leukemia group. In the past three years I have studied five leukemias, each of whom had at sometime one or more foci of infection caused by hemolytic staphylococcus aureus. Three of these cases were studied to see if the white cell count and the number of immature cells bore any relationship to the infections. Two of these cases were given antigen made from staphylococcus aureus, in the one case autogenous vaccine and in the other commercial staphylococcus toxoid. In each case the white cell counts were low at the start and as the doses of antigen increased, the level

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The staphylococcus toxoid was used in immunizing doses on a fourth case of leukemia without infection, in which the white blood cell count was controlled with Fowler's solution. Here again the white blood cell count increased and immature cell forms were found in the blood smear.

The data is too meager to state that infection in these cases played any definite rôle in inducing or aggravating the leukemic process.

*Erythrocyte Sedimentation. Clinical Significance and Evaluation as a Quantitative Procedure.* By THOMAS HALE HAM (introduced by Clark W. Heath), Boston, Mass.

Variation in the stability of erythrocytes in suspension was found to be a non-specific phenomenon which was altered by: plasma fibrinogen, serum globulins, lipoids, viscosity, hematocrit, mean corpuscular volume of erythrocytes, anticoagulant, diameter of sedimentation tube and height of blood column. The sedimentation rate was a measure of the degree of erythrocyte instability and, as such, had no direct clinical importance. The clinical interpretation of the sedimentation rates depended upon the significance of the abnormal blood constituent, and not upon the altered suspension stability.

Employing the Rourke-Ernstene technique, 260 determinations of the corrected sedimentation "index" on 65 subjects showed linear correlation with the concentration of plasma fibrinogen only when there were normal values for serum globulins and in general for lipoids. The sedimentation rate of erythrocytes in defibrinated blood was observed with correction for variations in hematocrit in 72 subjects. These rates were extremely slow and almost a constant in normal subjects; they were slightly accelerated and variable in abnormal subjects with normal serum globulins and lipoids; but were strikingly elevated in instances of increased serum globulins or lipoids. The corrected defibrinated sedimentation rate showed no relation to the concentration of plasma fibrinogen.

The sedimentation techniques of Wintrobe, Westergren, Linzenmeier and Cutler produced less satisfactory correlation with plasma fibrinogen than the method of Rourke and Ernstene. However, none of the above techniques using whole blood, nor the technique employed

here for defibrinated blood, could be used as an accurate or specific method of estimating the concentration of any one blood constituent, such as plasma fibrinogen, serum globulin or lipoids, because of the influence of variations in any one of these substances on the suspension stability.

*Neutropenia (Agranulocytosis). Fatigue as an Etiological Factor and Monocytosis as a Prognostic Sign.* By PAUL REZNIKOFF, New York, N. Y.

Of the four etiological factors important in the causation of neutropenia—fatigue, drugs, menstruation and infection—fatigue previous to the onset of illness was found to be the most prominent predisposing factor in 11 of 13 patients. No inquiry was made into this feature in the 2 remaining cases. Drugs known to be of etiological significance in this disease were taken in 9 cases; in 3, no history of their use could be elicited. One patient who had never taken drugs before her attacks received 1.62 grams of amidopyrine as a sedative during her fourth attack and recovered. Menstruation was a factor in 3 of the 9 female patients; 6 had passed the menopause. Infections were of possible importance in 6 patients; in 4, no history of infections could be elicited, and in 3, no previous infection could be found.

The most constant hematological sign of recovery was a pronounced monocytosis which usually appeared before any other evidence of improvement. Only a sporadic or slight monocytic response was present in the 3 fatal cases.

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From a study of the rate of blood formation in over 600 patients with various types of anemia, it is concluded

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From a study of the rate of blood formation in over 600 patients with various types of anemia, it is concluded



that the increase or decrease in the erythrocyte count per week ( $It$ ) is equal to the difference between the number of new erythrocytes formed and the number destroyed ( $P-D$ ) in a given weekly period ( $t$ ). For pernicious anemia the weekly rate of destruction ( $D$ ) =  $0.22 \div (0.176 \text{ times } Eo$ , the initial red blood cell count). In normal individuals, in certain anemias (acute hemorrhage, iron deficiency, pernicious anemia during remission) and for transfused normal erythrocytes,  $D = 0.22 Eo$ . The average period of erythrocyte survival in pernicious anemia in relapse varies from 1.6 to 4.54 weeks, depending on the erythrocyte count. In the other conditions mentioned, the average duration is 4.54 weeks.

The theory is advanced that erythrocytes, being no longer living nucleated cells, persist "indefinitely" unless they are fortuitously destroyed by trauma. "Wearing out" then, is not in the order of "age," but in degree of trauma. Modifying factors are (a) total erythrocyte concentration, (b) shape, (c) size, (d) elasticity, (e) nature of the capillary bed, (f) velocity of the stream, and (g) chemical environment. Comparable tissues in this respect are the hair and nails.

*The Effect of Intramuscular Administration of a Globulin Substance Derived from Normal Human Plasma in Hemophilia.* By FREDERICK J. POHLE and F. H. L. TAYLOR (introduced by George R. Minot), Boston, Mass.

Patek and Taylor<sup>1</sup> have demonstrated the effectiveness of a single intravenous injection of a globulin substance derived from normal human plasma in reducing the coagulation time of the blood in hemophilia.

The present observations demonstrate that the globulin substance may be given intramuscularly with similar effectiveness. After either single intramuscular or intravenous injection there was a prompt reduction in the coagulation time followed by a gradual rise with return to the pre-injection level in 24 hours.

When injections were repeated by the intramuscular or intravenous route within 7 hours, it was not possible to maintain the coagulation time at the reduced level. This refractory period does not last longer than 24 hours after the last injection.

However, during this refractory period the plasma or blood of the injected hemophilic patient showed no loss of clot-promoting power when measured *in vitro* against a second control hemophilic blood. Therefore, the refractory phase in the injected patient cannot be explained on the basis of a deficiency of globulin substance alone.

*Experiences with Insulin in Non-diabetic Individuals.* By REGINALD FITZ and (by invitation) BEN VIDGOFF, Boston, Mass.

A series of non-diabetic individuals were given ordinary glucose test meals with and without added insulin. Three types of curve were encountered. In the one,

insulin had its usual effect in lowering the blood sugar concentration after a test meal. In another group, no effect of insulin was demonstrable, the curves being essentially the same with and without insulin. In the third group of cases, the glucose tolerance after the administration of insulin appeared diminished; so that the resultant curve following insulin was a diabetic-like curve, whereas without insulin, the curve was such as is obtained in normal individuals after a glucose meal. That insulin in normal individuals under certain circumstances may appear to lower sugar tolerance already has been recognized. The reason for this finding is not altogether clear. In our experience, the activity of the thyroid gland is one certain factor. The size of the dose of insulin used is another factor in determining the type of sugar tolerance test obtained. Possibly the activity of the anterior lobe of the pituitary gland is a third factor, though our data on this score are not altogether convincing.

*Parathyroid Diuresis.* By CLARENCE L. ROBBINS (introduced by John P. Peters), New Haven, Conn.

Recent studies of the effect of injections of parathyroid extract on the excretion of electrolytes in urine indicate that the excretion of base and particularly sodium is increased in the first few hours after injection. Ellsworth and others had previously demonstrated the increased rate of excretion of phosphorus immediately following injection of parathyroid extract.

In the studies here reported an attempt was made to determine whether the parathyroid extract specifically influences the excretion rate of sodium and of phosphorus or whether the observed increases are simply a resultant of increased renal filtration. Human subjects who presented no evidence of renal dysfunction were used for control observations and for studies of excretion rates of sodium, chloride; phosphorus, pH changes and exogenous creatinine clearances before and after injection of parathyroid extract.

In the first hour and continuing for three hours after injection a significant rise in pH of urine and in the excretion rates of water, sodium and phosphorus occurred. Creatinine clearances remained unchanged. Chloride excretion was not significantly influenced.

It is concluded that parathyroid extract exerts a diuretic action by inhibiting tubular reabsorption and not by increasing glomerular filtration as measured by creatinine clearances. Molecular ratios of excess sodium to phosphorus greater than 15 suggest that phosphatemia, immediately following parathyroid extract may be entirely secondary to diminished reabsorption of sodium by the renal tubules.

*Supra-Diaphragmatic Splanchnic Resection for Essential Hypertension: A Two Year Study of Results, Selection of Cases, and Physiological Considerations.* By REGINALD H. SMITHWICK (by invitation) and ROBERT S. PALMER, Boston, Mass.

In thirty-four early or moderate cases of essential hypertension followed from a few months to two years,

<sup>1</sup> Patek, Arthur J., Jr., and Taylor, F. H. L., J. Clin. Invest., 1936, 16, 113.

a fall of blood pressure to within normal limits in two-thirds, and almost to normal in the remaining third occurred.

There is marked symptomatic improvement in all. No marked fall in blood pressure is obtained in cases of malignant hypertension and only a moderate fall in 50 per cent of the cases of late hypertension. Symptomatic improvement of moderate or marked degree is obtained in all of late benign essential hypertension, and likewise in all of 6 cases diagnosed malignant hypertension. Medical treatment causes a substantial fall in the blood pressure of over 50 per cent of mild and moderate cases, while 90 and 75 per cent respectively, either have no symptoms or are very much relieved. Medical treatment causes a substantial fall in the blood pressure in one-third of the late cases, and there are no symptoms, or symptomatic relief is obtained in slightly less than 50 per cent.

Experiments on the blood flow in the arm after novocaine block, indicate that vasomotor control is much diminished. We suggest that the basis of essential hypertension may be at first a vasomotor instability, gradually becoming conditioned to a state of "chronic emergency." By sympathectomy of a large area of the minute vessels the first barrier of the peripheral resistance is lowered. It is not expected that the capillary resistance depending on tissue needs is affected by this procedure. In the early vasomotor stage essential hypertension may be halted at its inception. Later it may be relieved by abolishing the variability so characteristic of essential hypertension, but it is unlikely that the level can be lowered to normal after arteriolar change resulting from long continued hypertension has taken place.

*The Treatment of Polycythemia Vera by the Production of a Chronic Iron Deficiency State.* By WILLIAM DAMESHEK and (by invitation) HENRY H. HENSTELL, Boston, Mass.

The treatment of polycythemia vera is eminently unsatisfactory. Phenylhydrazine is exceedingly difficult to control and probably damaging to the liver. Fowler's solution is not well tolerated. X-ray therapy has been disappointing. Occasional venesections apparently stimulate blood production.

In 5 well-controlled cases previously treated with the above methods, a state of chronic iron deficiency was induced by multiple venesections and a diet grossly deficient in iron. Venesections, usually 6 to 8 in number, of 500 cc. were done twice weekly until the hemoglobin reached about 70 to 80 per cent and the erythrocyte count about 5.0 million. The daily dietary iron content was reduced to less than 6 mgm. Frequent studies were made of the clinical status, the blood, hematocrit, blood volume, and viscosity.

With this method, the patients were maintained symptom-free from 6 to 9 months, and there have occurred: great reduction in the various features of the plethoric state, relatively low hemoglobin concentration with very

low color index and mean corpuscular hemoglobin concentration, low hematocrit with low mean corpuscular volume and small average red cell diameters, and reduction in the blood volume and blood viscosity to normal values.

Rising values after 6 to 9 months have been readily controlled by a few venesections.

*A Delayed Disturbance of Nitrogen Metabolism Following Certain Infections.* By HAROLD A. BULGER, St. Louis, Mo.

The mysterious relationship of infection to such local disturbances as arthritis or nephritis inspires an interest in any correlated metabolic changes. We have noted certain striking phenomena following some acute infections. They have occurred after an intervening period of one to several weeks of seemingly normal metabolism. The most notable change has been an extraordinary excretion of amino nitrogen. There has been at the same time a reversion to a negative nitrogen balance. Most cases of nephritis have shown a more or less marked increase in amino nitrogen excretion. Even more striking has been a great increase in the output of amino nitrogen of certain cases of arthritis. The magnitude of this change may exceed ten times the usual amounts. We have noted an immediate fall after removal of foci of infection. It should be noted that as a rule this phenomenon is not associated with any febrile reaction.

*High Peripheral Venous Pressure. Its Lack of Relationship to Orthopnea and Dyspnea in the Absence of Congestive Failure.* By EUGENE B. FERRIS, JR., and (by invitation) JOHNSON MCGUIRE, Cincinnati, Ohio.

There is considerable clinical and experimental evidence that the dyspnea and orthopnea of cardiac failure are not related to increased venous pressure. However, a number of observers have maintained that such a relationship does exist, either directly through the effect of high venous pressure itself; or indirectly through stagnation anoxemia which is assumed to occur with high venous pressure, or through the associated increased intracranial pressure.

We have observed thirteen patients having extremely high venous pressures in the upper part of the body resulting from superior vena caval obstruction and have been impressed by the absence of dyspnea or orthopnea in the majority of such patients. Blood gas studies of blood obtained from the internal carotid artery and from the internal jugular vein, spinal fluid pressures, simultaneous cubital and femoral venous pressures, and clinical observations have been made on five consecutive patients having superior vena caval obstruction.

Dyspnea and orthopnea were absent in all five patients, although venous pressures and spinal fluid pressures as high as 50 cm. of water were recorded. The cerebral blood flow was diminished in some cases and normal in others. Stagnation anoxemia, when present, was not accompanied by respiratory symptoms.



*A Method for Determining the Minimal Infective Dose of Treponema Pallidum in Experimental Syphilis.* By HUGH J. MORGAN and (by invitation) GEORGE P. VRYONIS, Nashville, Tenn.

Our method offers a means of quantitating the dose of virus necessary for establishing syphilis in the rabbit. At least, one can say how many visible organisms are in an inoculum which produces an infection. The results so far indicate that a certain minimal number are necessary before infection will occur. This is a matter of interest since many experiments in rabbit syphilis are based on the assumption that, if *any* organisms are present, an infection will occur. Moreover, the method may be of some value in considerations of the question of a ultramicroscopic virus in syphilis.

*The Combination of Oxygen and Hemoglobin in the Blood of Patients with Liver Disease.* By ANCEL KEYS (by invitation) and ALBERT M. SNELL, Rochester, Minn.

The occurrence of anoxemia in advanced hepatic dis-

case has been reported previously, the available evidence indicating that ordinary pulmonary and circulatory factors are not responsible. Oxygen dissociation curves of the blood, calculated on the basis of an assumed pH, indicated a displacement of the curve to the right in some of these cases. The present study confirms the presence of oxygen unsaturation of the arterial blood in advanced cirrhosis. A study of the oxygen-hemoglobin combination in such cases, with measurement of  $pH_a$  and  $pH_v$  of the arterial blood as drawn and as equilibrated, shows an apparent decreased affinity of hemoglobin for oxygen. This decreased affinity persists over a range of from 30 to 80 per cent saturation and is sufficient to account for the observed anoxemia. The presence of hyperbilirubinemia alone is not a factor.

A study of laked blood, as contrasted to whole blood, in these cases, provides the necessary data to discriminate between alterations in the hemoglobin itself and abnormalities in the equilibrium across the cell membrane. The evidence obtained to date indicates that the hemoglobin itself, as in all pathological conditions which have been studied, is not physiologically altered.

# CHANGES IN THE VASOMOTOR REACTION ASSOCIATED WITH GLOMUS TUMORS

By SAMUEL J. STABINS, JOHN J. THORNTON AND W. J. MERLE SCOTT

(From the Department of Surgery, The University of Rochester, School of Medicine and Dentistry, Rochester, New York)

(Received for publication March 11, 1937)

The interesting clinical syndrome produced by tumors of the glomeric structures in the extremities was first described by Masson in 1924 (1) and called by him, glomus tumors. We have collected from the literature seventy-four cases of this condition (Table I). This condition is easily

record studies in the derangement of the vasomotor mechanism which is one of the outstanding characteristics of the disease. The latter has been recognized, but its nature has not been carefully investigated previously.

The following two cases have been seen in this clinic.

TABLE I  
Cases reported

Author	Year reported	No. of cases	Sex	Location	Duration
Masson	1924	3	2 females 1 male	Subungual Finger	5 to 30 yrs.
Martin-Dechaume	1925	2	Females	Subungual	— to 25 yrs.
Masson-Gery	1927	4	3 males	—	—
Prodanoff	1927	1	Male	Thigh	13 yrs.
Nicod	1927	1	Female	Subungual	4 yrs.
Bonnet	1927	1	Female	Subungual	36 yrs.
Greig	1928	2	Male Female	Arm Calf	10 yrs. 8 yrs.
Lortat-Jacob and Brosse	1928	1	Female	Subungual	16 yrs.
Janischewski-Lebel	1928	1	Female	Finger	18 yrs.
Hopf	1930	4	2 males 2 females	—	4 to 16 yrs.
Picard	1931	1	Male	Subungual	Long time
Dupont	1931	2	1 female	—	—
Fernández and Monserrat	1931	1	Male	—	—
Cascos-Costen	1932	1	Female	Arm	10 yrs.
Paulian, Popescu, Marinesco-Slatina	1933	1	Female	Rt. arm	Long time
Adair	1934	10	4 females 6 males	4 subung.	Av. 9 yrs.
Mason-Weil	1934	1	Male	Lt. knee	37 yrs.
Oughterson	1934	1	—	Lt. thumb	—
Stout	1935	11	6 females 5 males	4 subung.	1 to 30 yrs.
Bailey	1935	7	—	2 subung.	4 to 20 yrs.
Love	1935	1	Male	Lt. elbow	—
Lewis-Geschickter	1935	17	10 females 7 males	Arms and legs	— to 15 yrs.
		74 cases			

diagnosed by anyone conversant with the condition but, according to the experience of Lewis and Geschickter (22), the clinical diagnosis is frequently not made. The recent literature contains several excellent clinical and pathological studies of the condition (3, 4, 5). We wish to add two typical cases with the characteristic symptomatology and histological appearance. Our purpose is not one to add to the limited number of cases so far reported in the literature but to re-emphasize the significance of the lesion and particularly to

## CASE REPORTS

*Case I*, Number 95098, married, white female, aged 64 years; first seen in the surgical clinic on October 23, 1934. Chief complaint—Injury to the left thumb. Present illness—Caught left thumb in a door this morning. She has had a red spot on the volar surface of the thumb for twenty-five years. It is very sensitive to the slightest trauma and causes referred pain over the hand and up the arm. Physical examination—Negative findings except for the left thumb. There is no appreciable swelling but on the volar surface, distal phalanx of the left thumb, there is a small dark purplish-red area about 1 cm. in diameter which is extremely sensitive to touch. There is some localized vasodilatation around this area associated with increase in surface temperature and local perspiration. A diagnosis of glomus tumor was made by Dr. Thornton. She was given 50 mgm. hours of radium unfiltered to this area, partly as a therapeutic test to verify the diagnosis of glomus tumor. She failed to respond as is the rule with this lesion, thus making the diagnosis of glomus tumor even more certain. On November 23, a local excision of the tumor was made under Evipal anesthesia. Prior to and after excision, temperature readings were made of both hands and forearm. Complete relief of excruciating pain which had been present on tactile stimulation for twenty-five years was obtained by simple excision locally of the intact tumor. Histological studies revealed the characteristic appearance of a glomus tumor.

*Case II*, Number 84682, married, white female, aged 23 years, seen in the surgical outpatient on January 18, 1935, because of melena. During routine physical examination, a pea-sized purplish-red, extremely sensitive tumor was noted on the terminal phalanx of the second left digit. It was just under the base of the nail and on its inner aspect. She states that it is extremely sensitive to touch. She does not remember how long she has noticed this lesion, but feels sure it is at least fifteen years. She had consulted several physicians in years past regarding this pain but had given up hope of relief as the remedies suggested were ineffective. The bleeding per rectum was accounted for on the basis of internal hemorrhoids. On

*A Method for Determining the Minimal Infective Dose of Treponema Pallidum in Experimental Syphilis.* By HUGH J. MORGAN and (by invitation) GEORGE P. VRYONIS, Nashville, Tenn.

Our method offers a means of quantitating the dose of virus necessary for establishing syphilis in the rabbit. At least, one can say how many visible organisms are in an inoculum which produces an infection. The results so far indicate that a certain minimal number are necessary before infection will occur. This is a matter of interest since many experiments in rabbit syphilis are based on the assumption that, if any organisms are present, an infection will occur. Moreover, the method may be of some value in considerations of the question of a ultramicroscopic virus in syphilis.

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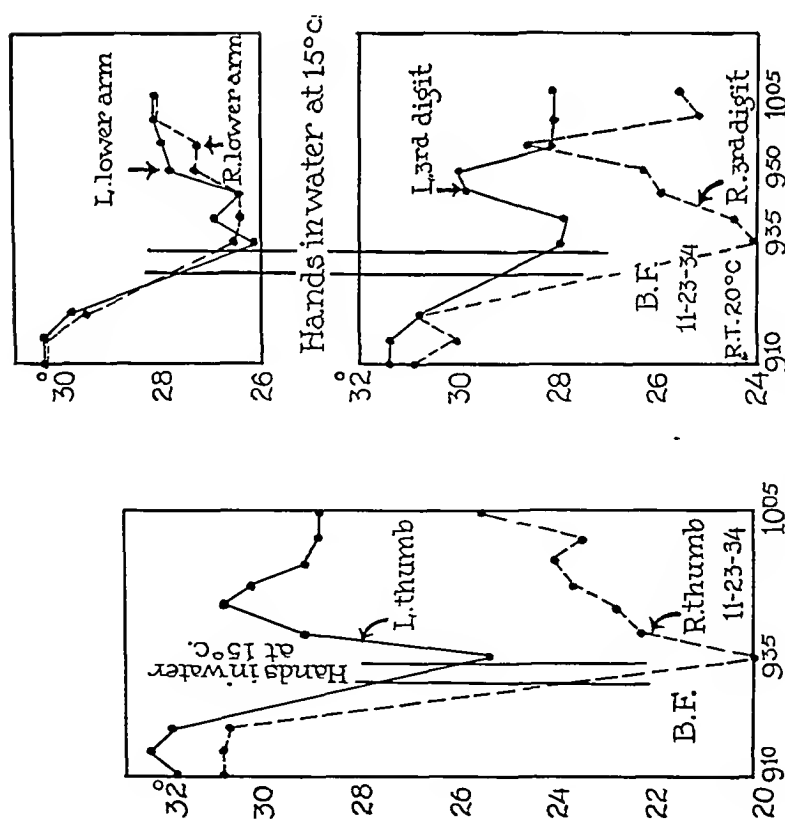


FIG. 2. CASE I (B. F.). TEMPERATURE READINGS TAKEN JUST PRIOR TO REMOVAL OF TUMOR

The involved thumb is slightly warmer than the uninvolved but after immersion into water for five minutes at 15° C., the recovery on the affected side is almost immediate with a difference varying from six to eight degrees. An almost similar picture is seen in the third digit. The temperature changes in both forearms remain about equal.

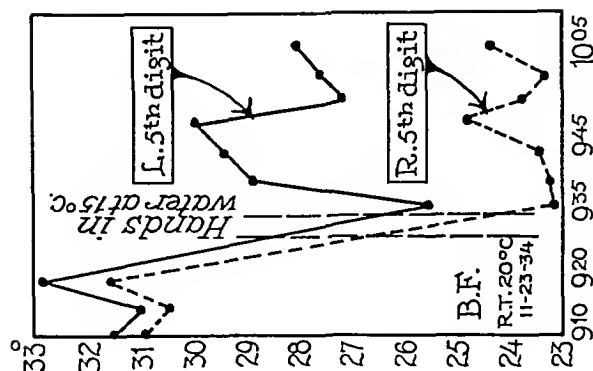


FIG. 3. CASE I (B. F.)

Shows a similar recovery in the fifth digit with a difference varying from six to eight degrees.

February 15, 1935, excision was performed under gas-oxygen. Histological studies revealed the characteristic appearance of a glomus tumor.

The clinical pictures of these two cases are typical. The outstanding characteristic of the lesion is pain usually of a peculiarly excruciating quality produced by the lightest pressure. In both cases the patients had had the lesions for years and had consulted many doctors without obtaining relief. In the early years the pain was more localized to the site of the lesion or the digit involved but later the pain was projected farther up the ex-

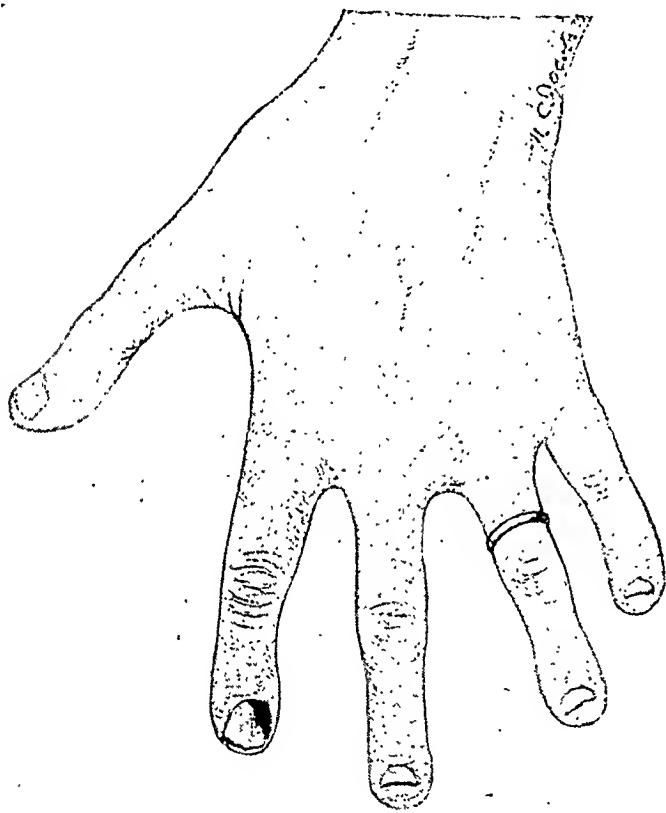


FIG. 1. CASE 2, SHOWING THE DISCOLORATION OF THE TUMOR BENEATH THE NAIL OF THE INDEX FINGER

tremity as well. Both patients had noticed an increased warmth in the involved region. The lesions had the characteristic purplish coloration, they were from five to eight mm. in diameter; in one case the tumor was on the volar surface of the distal phalanx of the thumb, the other one was under the nail of the left index finger near its base. In the latter case the thickness of the nail was definitely diminished over the lesion, its surface was bulged upward by the tumor and the purple discoloration could easily be seen through the nail (Figure 1).

Several authors in discussing the richness of nerve fibrils found in the glomus tumors have suggested their relationship to the pain on the one hand and the vasomotor system on the other, but no accurate studies of the vasomotor mechanism involved have as yet been recorded. We have knowledge of a number of pathological conditions which cause abnormal vasoconstriction but those which produce vasodilatation are less well known.

#### OBSERVATIONS

The following procedure was used in both cases in recording the vasomotor changes. The temperatures of several digits and of the forearms were taken at five minute intervals; all readings were recorded in the constant temperature room. After obtaining a base line in the initial readings, both hands were immersed in cold water at 15° C. for five minutes. After quickly drying the hands without rubbing, the temperature readings were resumed and the rate at which the different points recovered from the cold was recorded. Similar observations were made at various times after operation.

The normal response following this standard cooling in water is a gradual reheating of the hand the speed of which varies somewhat in different individuals and according to the physiological condition of the individual but which is always synchronous on the two sides. In the presence of a glomus tumor, there was regularly an asymmetrical response. Frequently, the initial temperature level and the minimum temperature produced by cooling were higher on the involved side than on the opposite one. But whether or not this was true, a very rapid reheating of the hand was always observed on the affected side; within ten minutes there was a difference of six to eight degrees between the affected hand and the uninvolved one. Also, it was noted that this evidence of vasodilatation was not limited to the affected digit nor to any peripheral nerve field but covered the entire hand. Thus in both cases the palmar surface of the fifth finger (ulnar nerve) responded in practically the same manner as the involved and contiguous digit (median nerve). After the removal of the glomus tumor, the abnormal vasodilatation eventually disappeared completely as measured by the response to this standard test and the two sides became symmetrical as

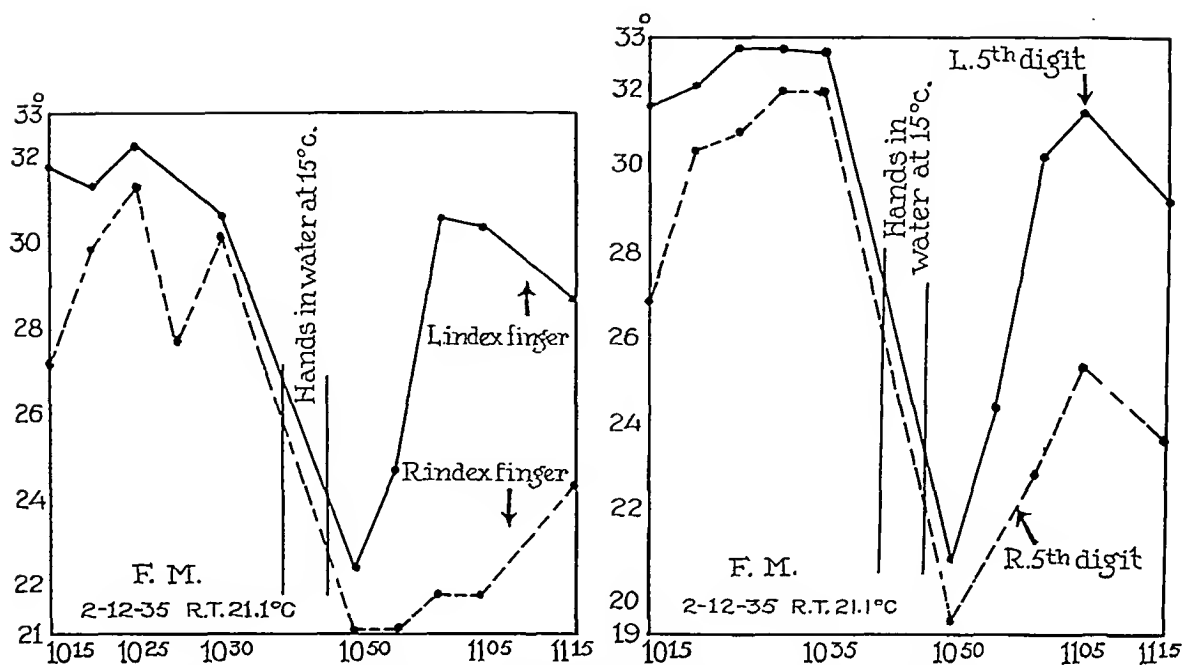


FIG. 6. CASE II (F. M.). TEMPERATURE READINGS TAKEN PRIOR TO OPERATION

The changes are similar to those recorded in Case I. The lesion is located on the left index finger but here again, the fifth digit presents the striking vasomotor changes.

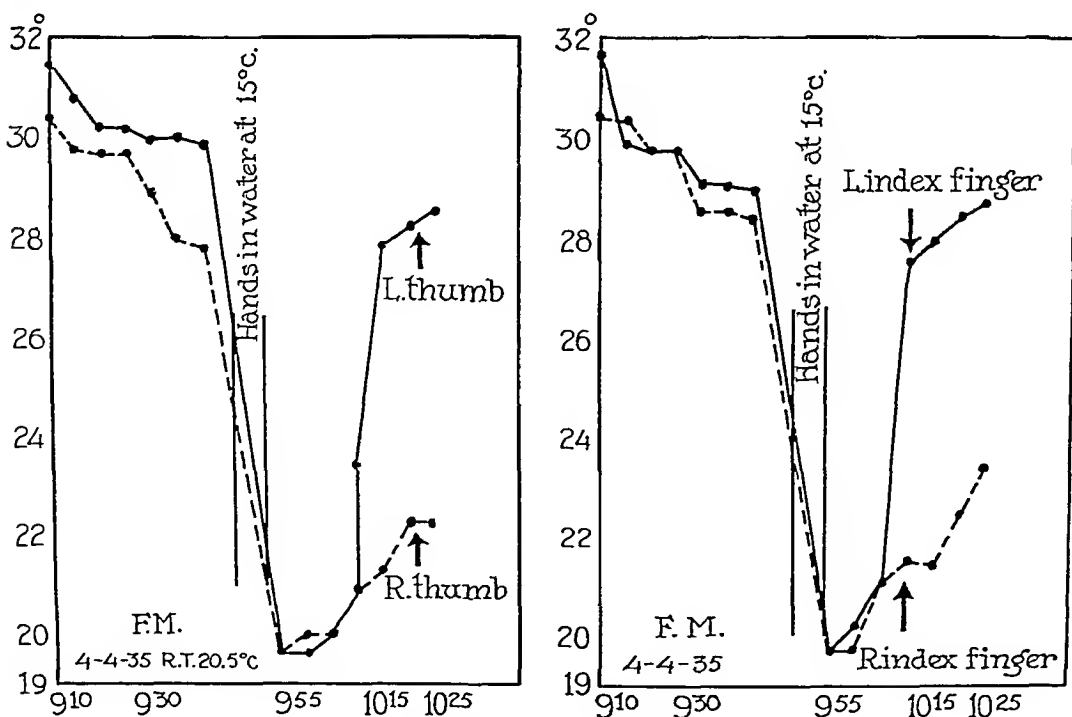


FIG. 7. CASE II (F. M.)

Almost two months after removal of the tumor, there is still evident the disturbance in the vasomotor mechanism. The patient still complained of a dull ache over the scar, and there was slight tenderness to pressure.

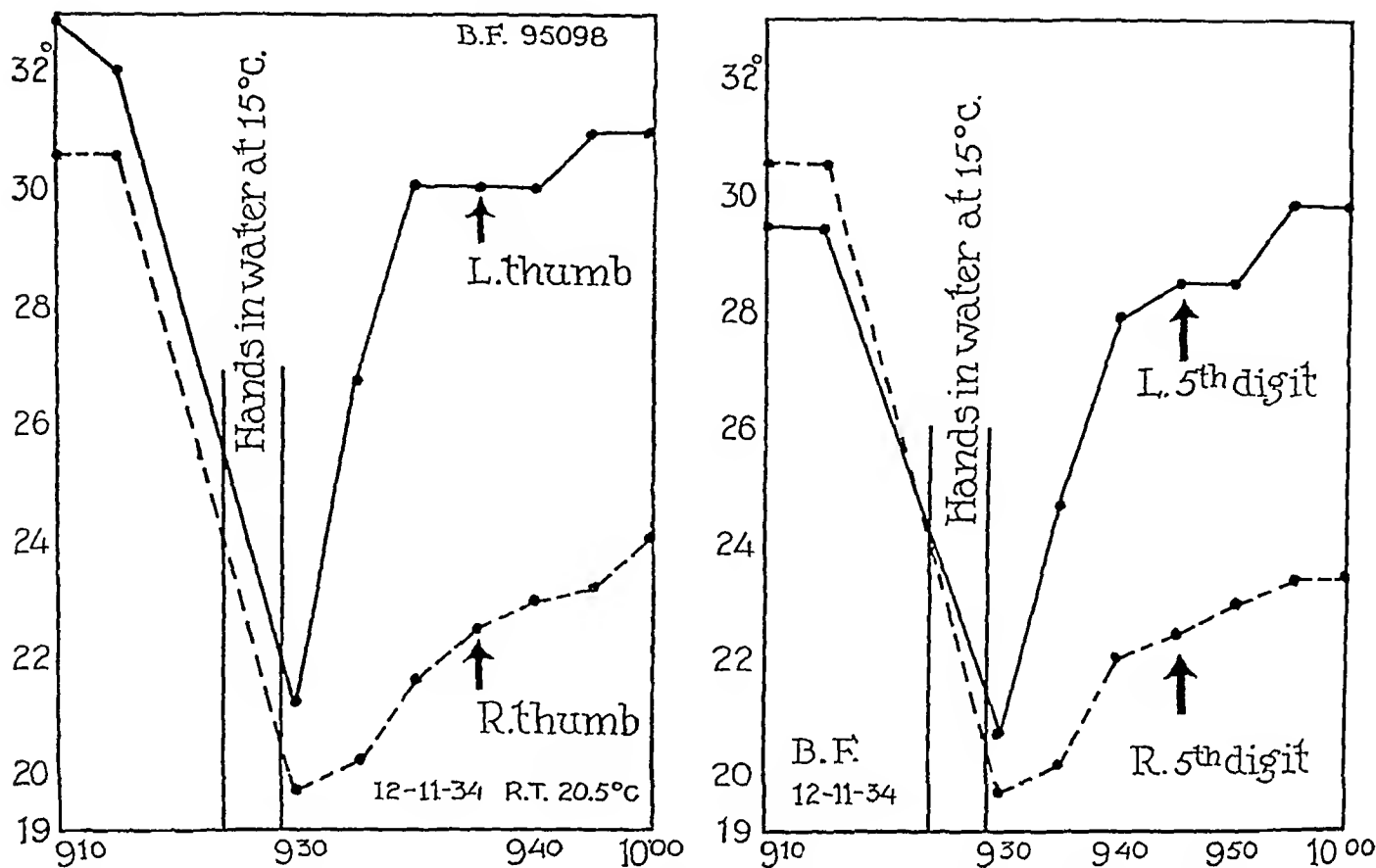


FIG. 4. CASE I (B. F.). TEMPERATURE READINGS TAKEN THREE WEEKS AFTER OPERATION

The vasomotor disturbance is still present. At this time, the patient still had a dull ache over the scar and there was slight tenderness to pressure. These temperature changes lasted for about three months at which time all symptoms had completely disappeared.

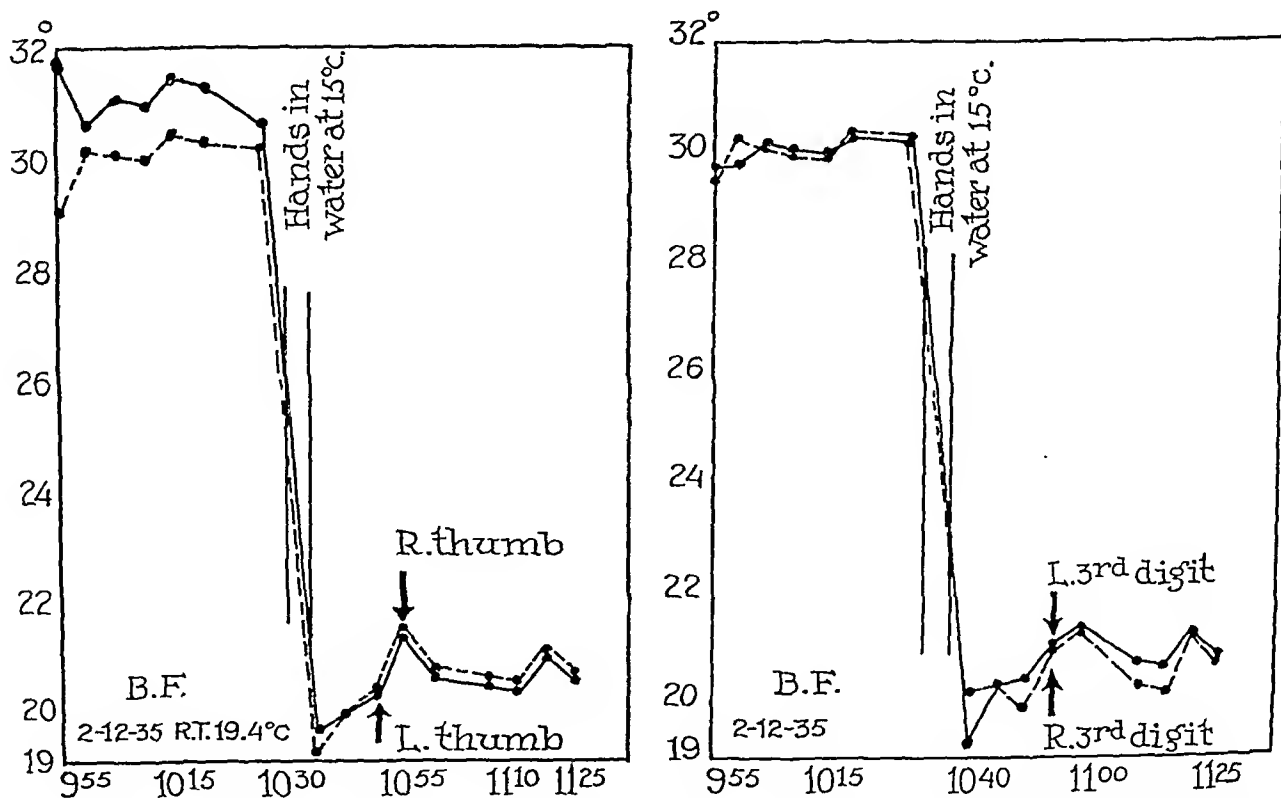


FIG. 5. CASE I (B. F.). TEMPERATURE CHANGES TAKEN THREE MONTHS AFTER OPERATION SHOWING A UNIFORM RECOVERY ON BOTH SIDES

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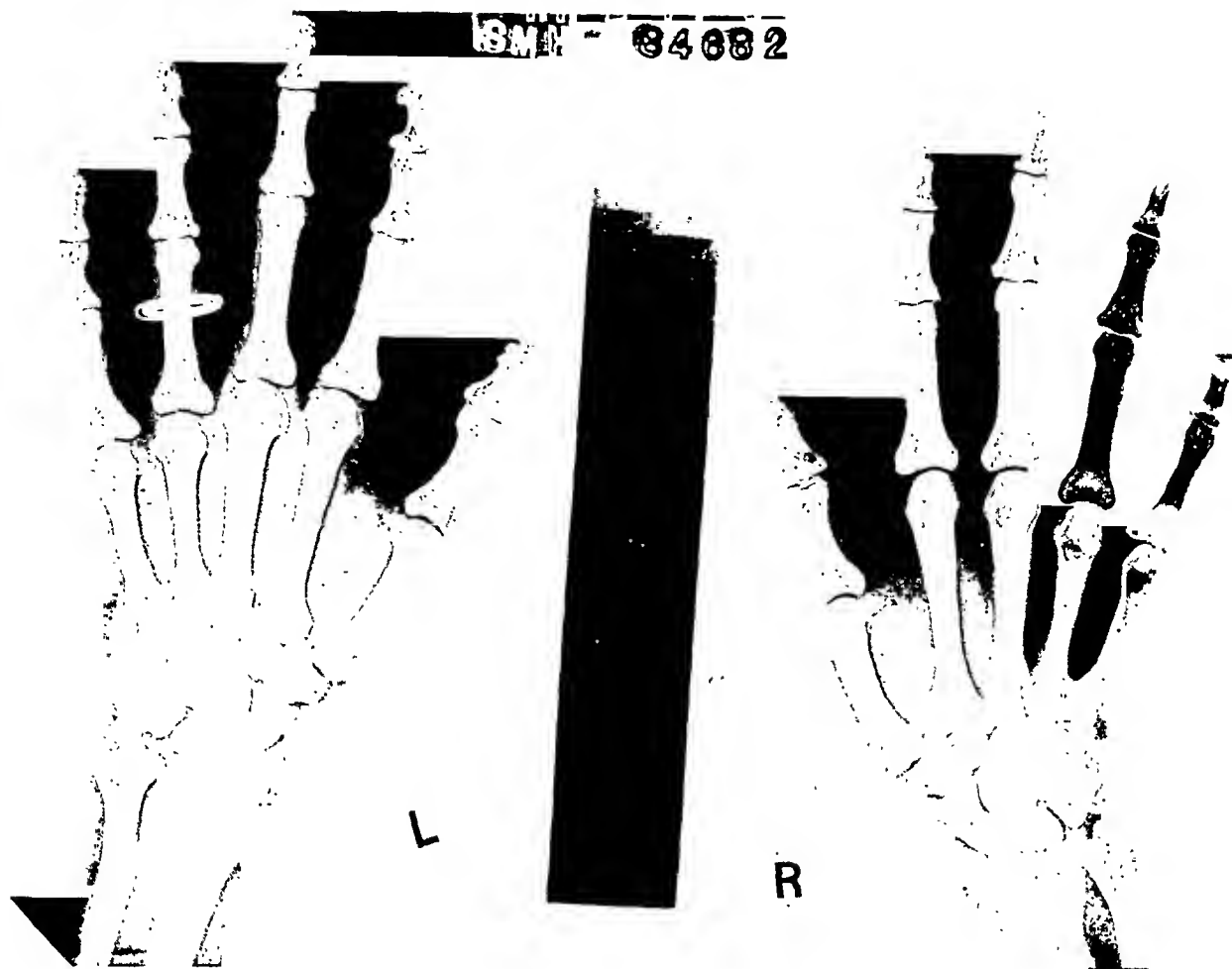


FIG. 10. PHOTOGRAPH OF CASE II

There is noted some thinning out of the shaft on the left index finger due to local pressure but no generalized changes that usually accompanies painful osteoporosis.

any tendency towards a spotty osteoporosis such as characterize reflex traumatic arthritis. This same observation that persistent dermal vasodilatation fails to produce osteoporosis has also been made in erythromelalgia by one of the authors with Dr. J. J. Morton. These considerations indicate either a dissociation of vasodilatation in the skin and in the underlying bone, or that vasodilatation is not a sufficient cause in and of itself to produce osteoporosis (Figures 9 and 10).

#### CONCLUSIONS

1. Two typical cases of glomus tumor are added to those already collected, and the importance of diagnosing this condition is reemphasized.

2. Studies of the vasomotor mechanism involved are recorded. The efferent side of the reflex is not limited to the peripheral nerve distribution of origin. The vasodilatation associated with

the tumors completely disappears after surgical removal.

3. The persistence of the vasodilatation for two to three months associated with discomfort in the scar suggests that pain acts as the afferent stimulus which produces the characteristic vasodilatation.

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# THE DAILY URINARY EXCRETION OF ESTROGENIC AND ANDROGENIC SUBSTANCES BY NORMAL MEN AND WOMEN<sup>1</sup>

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It is well established that normal men and women excrete estrogenic and androgenic substances in the urine. Qualitatively speaking, it is also well established that at least a part of the androgenic activity in men's urine is due to androsterone and dehydro-androsterone (1, 2, 3), but we do not know the exact chemical nature of the androgenic material in women's urine. Furthermore, we do not know the true chemical nature of the estrogenic substances found in the urine of normal men and non-pregnant normal women. Comparative biological and chemical studies by Dorfman, Gallagher and Koch (4) indicate that the estrogenic activity of men's urine is not theelin but very likely theelin.

Quantitative information on the distribution of these activities in adult human urine is even less satisfactory. In most cases the extractions have been incomplete, and the assays are based on too few animals or have not been properly compared with pure standards. The brief review below reveals the striking extremes reported thus far and the impossibility of interpreting most of the data in terms of common standards of known purity.

Funk and Harrow (5) employed concentrated alcoholic extracts from concentrated human urines and concluded that the urines from men 70 to 80 years of age do not contain enough androgenic material to be detected by the comb-growth method. Siebke (6) reported the daily excretion of 0 to 1 capon unit of androgens in young women's urine and that it requires 2.5 to 10 liters to yield 1 capon unit. He did not acidify the urine, and his extraction probably was very incomplete. Ogata and Ito (7) from studies by chloroform extraction of urine concluded that the excretion in normal man varies with the season. Bühler (8) prepared benzene extracts of the alkaline urine. He reports daily excretions of 0 to 2

capon units in normal men. His capon unit is approximately equal to 0.75 international androgenic unit or 0.075 mgm. of androsterone. Two recent studies give much higher yields on normal individuals. A study by Simpson, de Fremery, and Macbeth (9) by chloroform extraction reports the excretion of 10 to 50 capon units, equivalent to approximately 7 to 35 international capon units per day in normal women, but that the fluctuations bear no relation to menstruation. Kochakian (10) employed a modification of the method of Funk and Harrow (5). He reports the excretion of 19 to 48 international capon units per day in normal men.

There is somewhat better agreement on the daily excretion of estrogenic activity in women, but practically no data on the excretion in men. Siebke (6) first reported 0 to 180 mouse units per liter of women's urine throughout the cycle. From studies on eight normal women he concluded that there is a low excretion previous to and during menstruation. Later Siebke (11) reported 10 to 140 mouse units of estrogenic activity per liter of urine in women with a distinct rise between the 10th to the 15th day of the cycle. Frank (12) reported 20 to 320 mouse units per day with peaks at 12 to 15 days after the onset of menstruation and 4 to 8 days before the next bleeding. Bühler (8) reported 10 to 60 mouse units per day in normal men. Gustavson and Green (13) and Smith and Smith (14) also report two peaks in the excretion of estrogenic activity in women during the menstrual cycle. The former find 0 to 50 rat units per day with peaks at the 9th to 14th day and the 14th to 21st day after menstruation. Smith and Smith (14) record the peaks at 12 to 17 days after menstruation and during menstruation.

In this paper we present our methods of extraction and assay in detail and the results on four normal men and four normal women for 27 to 45 consecutive days. The male urine was collected

<sup>1</sup> These investigations were supported in part by a grant from the Rockefeller Foundation.



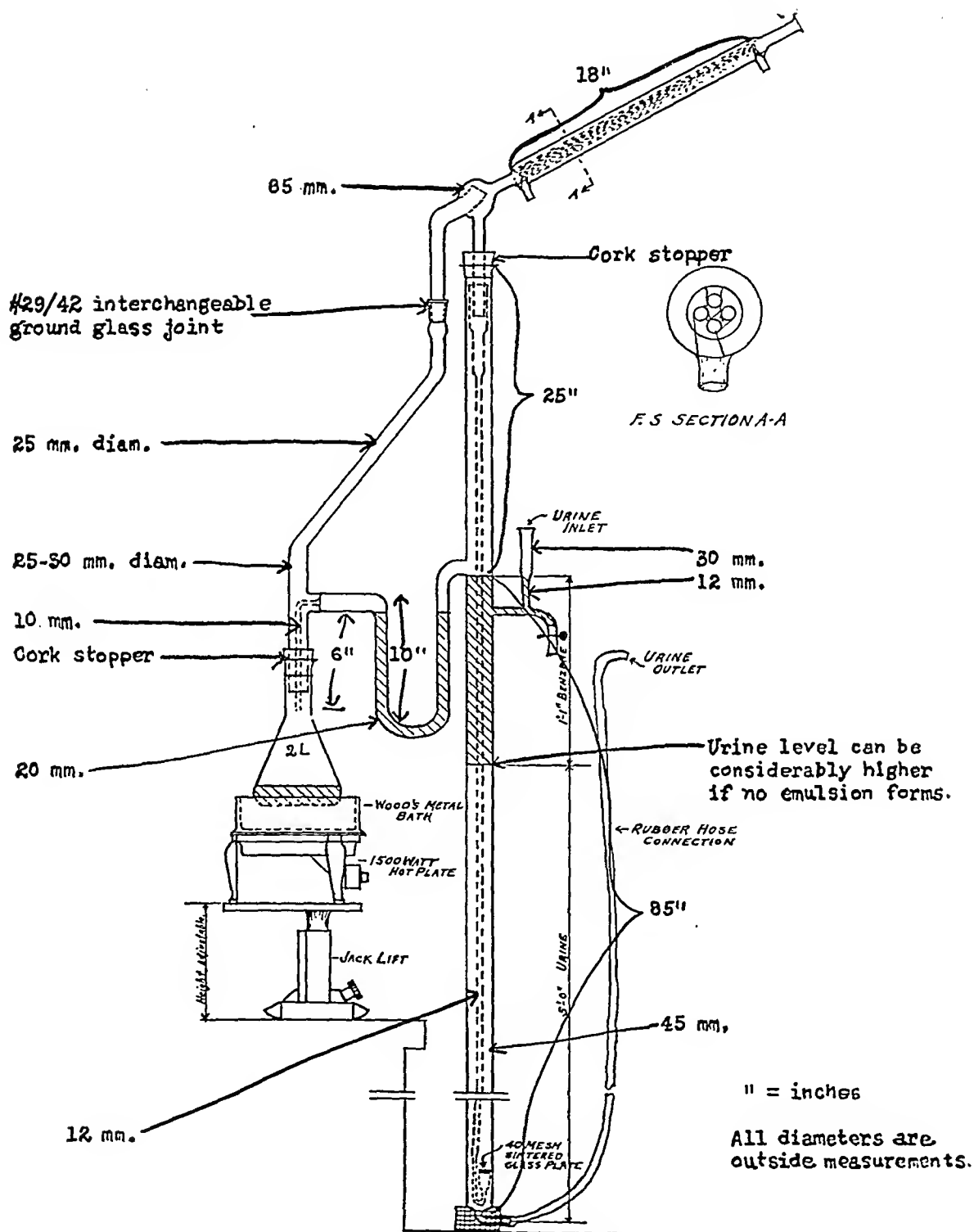


FIG. 1. CONTINUOUS EXTRACTOR FOR SEX HORMONES FROM URINE

in three-day samples with a few exceptions. The women's urine was collected in two-day periods during the cycle excepting during menstruation when it was collected as one sample. The women were found to be normal individuals by Dr. M. E. Davis of the Department of Obstetrics and Gynecology, University of Chicago.

#### EXPERIMENTAL

*Methods.* The evidence that our extraction of urine is complete is shown by Gallagher, Koch and Dorfman (15) and has also been confirmed repeatedly in our subsequent studies. The quantitative value of the male-hormone assay by the comb-growth method on the basis of the characteristic curve found by Gallagher and Koch (16) is also well established as a result of numerous quantitative studies. The estrogenic assay, in our opinion, is by no means as satisfactory because of the greater individual animal variations and the necessary limitation to the use of no more than ten spayed rats for each assay on account of the lack of material for injection.

*Extractor.* The special features of the extractor are the very rapid distillation of the benzene, the very fine division of the benzene, and the long column of urine through which the benzene rises. The condenser is very efficient because it is cooled by a current of water through the jacket and through the four inner tubes. These special features make the extraction very efficient and rapid. In place of Wood's metal, one may use a 50:50 mixture of lead and tin. The total height of the apparatus is eleven feet.

*Extraction of androgenic and estrogenic activities from urine.* The 24-hour or larger sample of urine is acidified by the addition of 100 cc. concentrated commercial HCl per liter of urine. After being boiled for 15 minutes under a reflux condenser and cooled, the urine is extracted in the special extractor with benzene until it has been contacted with at least twelve volumes of benzene. (Originally we boiled the urine 1 to 2 hours, but we now recommend 15 minutes because the yield of androgenic material is increased approximately by an average of 66 per cent (17).)

If the amount to be extracted is not greater than the capacity of the extractor (2000 to 2500 cc., depending on the extractor), the urine is transferred to the extractor, filling it to the mark 10 inches below the inlet (for smaller samples, water may be added to bring it to the mark), then enough benzene added at the urine inlet just to fill the U-tube connection to the flask. About 500 cc. of benzene are transferred to the flask which is connected with the apparatus. The benzene is heated to boiling, but it should be observed that when the benzene first begins to boil vigorously, there is some danger of flooding the condenser. If this happens, the hot plate should be lowered momentarily, then raised again and adjusted to a constant rate of boiling. After the boiling has continued for at least one hour, the rate at which benzene

is distilled over is determined by collecting the benzene from the small side-tube at the right-hand side. The rate of distillation should be 100 to 140 cc. per minute. The benzene collected in determining the rate should be returned at the urine inlet after closing the side tube. After the rate has been determined, the extraction is continued until at least twelve volumes of benzene per volume of urine have been passed through the urine. In the early part of the extraction, an emulsion may form at the surface of the urine. If this happens, the hot plate under the flask should be lowered for about one-half hour or until the emulsion is broken. If the emulsion persists, a small amount of saturated salt solution should be added at the urine inlet and allowed to stand for another half hour. If this fails, a few cc. of a 3 per cent solution of sodium glycocholate should succeed in breaking the emulsion.

If a continuous flow of urine is being extracted, the rate of introduction of urine at the inlet and the outflow at a constant level at the outlet must be so regulated that the urine is introduced at one-twelfth the rate of distillation of the benzene.

The benzene extract is evaporated to a viscid oil on the water bath or in a flask under diminished pressure to hasten evaporation. If this is attempted on a hot plate, there is danger of overheating. The oil is next dissolved in 50 cc. of ether and quantitatively transferred to a 250-cc. separatory funnel with three washings of ether of 15 cc. each. The ether solution is then shaken with three changes of 20 cc. of a saturated aqueous solution of sodium bicarbonate. The ether layer is quantitatively recovered. The aqueous phase is discarded. If it is not desired to separate the male and female hormones, the ether solution may be evaporated to the viscid oil stage, taken up in 95 per cent alcohol, filtered through paper into a 25- or 50-cc. volumetric flask, and diluted to the mark by washing through the filter with 95 per cent alcohol. Parts of this alcoholic stock solution are used for the assay as indicated under *Assay for estrogenic activity* below. It is better, however, to separate the androgenic and estrogenic activities before conducting either assay.

*Separation of the androgenic and estrogenic hormones.* The ether solution is shaken ten times with 50-cc. lots of 10 per cent NaOH in water, combined, and the NaOH washings saved. Then the ether layer is washed once by shaking with 15 cc. of 10 per cent  $H_2SO_4$  and washed again four times with 15-cc. volumes of distilled water. The acid and water washings are discarded. The combined alkaline-aqueous solution contains the estrogenic material; the ether layer retains the androgenic hormone. To recover the estrogenic substances, the alkaline-aqueous solution is acidified with 160 cc. 8 N  $H_2SO_4$ , allowed to cool, and tested with litmus to be certain that it is acid. The acidified aqueous solution is extracted with 150-, 100-, 75- and 50-cc. portions of ether. The aqueous acid solution is discarded. The combined ether extracts are shaken four times with 50-cc. portions of water. The ether layer is evaporated on a water or steam bath to a viscid oil. To remove the traces of water, about 15 cc. alcohol are added and the extract re-evaporated on a

(17). If, on the basis of other observations, we assume that 15-minute acid hydrolysis at boiling temperature before extraction would have increased the yield of androgenic activity by 66 per cent over the 2-hour boiling, the average values would be 63 to 69 international androgenic units, or the equivalent of 6.3 to 6.9 mgm. of androsterone per day.

The daily excretion of estrogenic material, expressed as theelin, varies from 2 to 29 gamma in the entire male group and even in one individual.

The average theelin values range from 9 to 12 gamma per day.

The rates of excretion of the androgenic and estrogenic activities bear no relation to each other. This is very well illustrated by the marked variations in the ratios obtained by dividing the androgenic units by the gamma of theelin per day. These are given as An/Es in Table II. However, the average values for the ratio are again remarkably constant, ranging from 3.3 to 4.6 on the 2-hour boiling treatment. If calculated on the

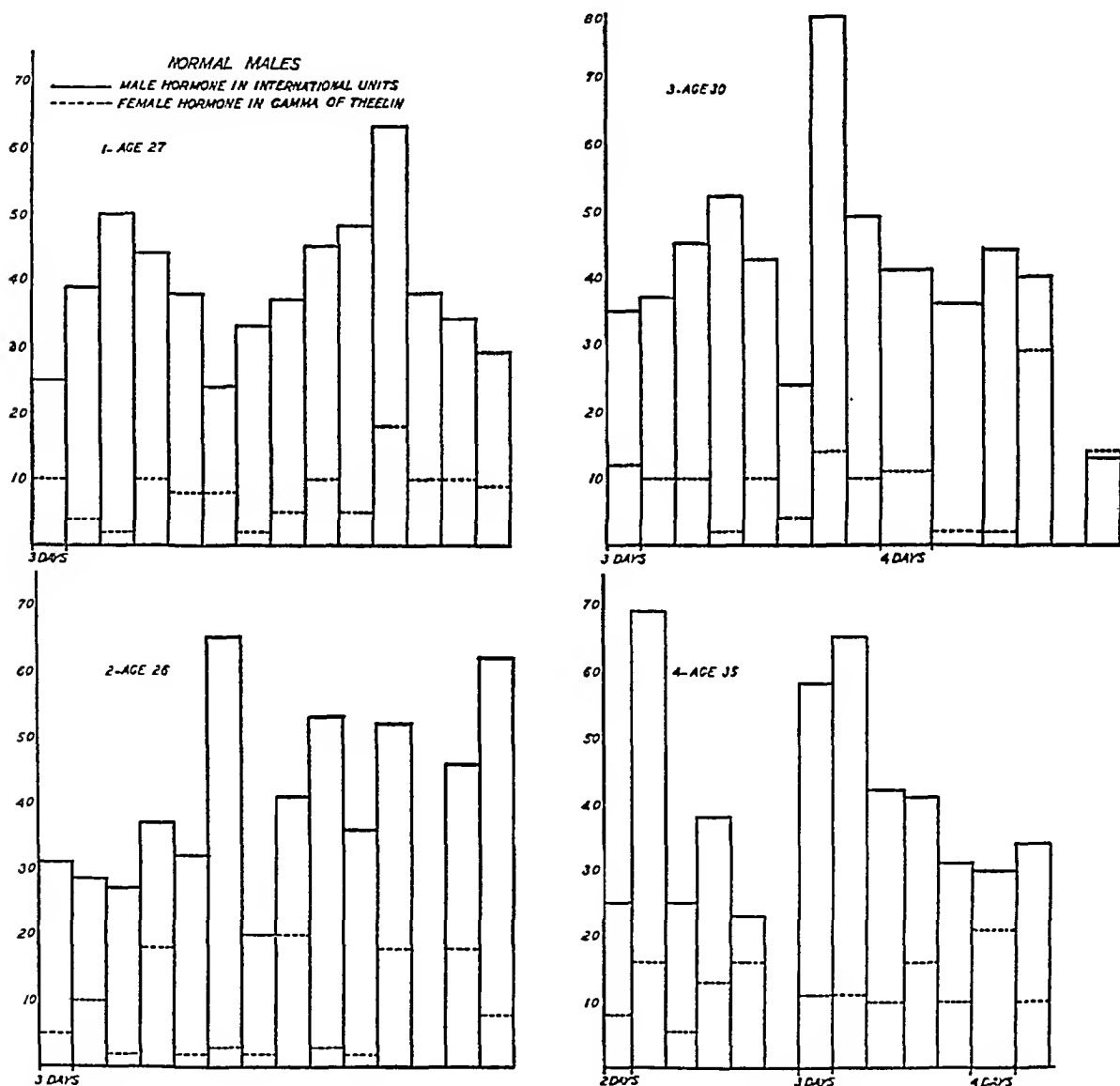


FIG. 2. THE EXCRETION OF ANDROGENIC AND ESTROGENIC SUBSTANCES IN THE URINE OF FOUR NORMAL MEN FOR PERIODS OF SIX WEEKS EACH

hot-water bath under diminished pressure (cautiously, to avoid loss due to foaming). The sample is made up to 25 or 50 cc. in a volumetric flask with 95 per cent alcohol. The solution is filtered while making up to volume. This serves as the stock solution for assay purposes. Aliquot parts are added to the desired volume of sesame or olive oil and the mixture heated under diminished pressure by immersion in a water bath until the alcohol is removed.

The ether layer containing the androgenic hormone is likewise evaporated and the residue dissolved in 95 per cent alcohol and made up to volume. A part or the whole may be dissolved in olive oil of desired volume.

*Assay for the estrogenic activity.* If the adult spayed albino rats have not been used for assay for two weeks previously, they must first be primed with a dose of 0.88 to 2.0 gamma theelin or the equivalent of estrogenic material ten days to two weeks before injection with the unknown preparation. The dose injected is always contained in 0.5 cc. of olive oil. Ten such primed rats are injected with 0.88 to 2.0 gamma of the standard theelin preparation and at the same time ten more are injected with the unknown preparation. Vaginal smears are made 42, 46 and 52 hours after the time of injection, examined under low power (100 $\times$ ), and the character of each recorded. The test is considered positive when the smear is free from leukocytes and contains aggregates of non-nucleated epithelial cells. From the percentage of animals showing a positive reaction, the concentration of estrogenic units is read from the standard curve of D'Amour and Gustavson (18) in terms of gamma of theelin as compared with the standard run in parallel.

*Assay of androgenic activity.* The androgenic activity of the alkali-insoluble fraction is determined by the method of Gallagher and Koch (16). Briefly, it consists of injecting seven capons daily for five days with 1 cc. each of the unknown in oil. The length and height of the combs are measured on the first and sixth day and the average total growth obtained. A standard containing 100 gamma of androsterone or its equivalent is assayed in parallel with the unknowns using the same number of capons. Since the growth obtained is a function of the initial comb-length, the average initial comb-length of the capons receiving the unknown preparations is corrected to the average initial comb-length of those receiving the standard preparation. For each millimeter difference in initial comb-length from the standard, there is a 0.2 mm. difference in total growth obtained on each unknown. Then, according to the curve obtained by Gallagher and Koch (16), the total capon units are determined. In place of the curve, Table I may be found more convenient for the calculation of the unknowns in terms of the standard. The footnote to the table illustrates the method of calculation.

#### DISCUSSION OF THE RESULTS

*Male urine.* The results on the four men are given in Figure 2 and Table II. The irregular

TABLE I

*Relation of increase in L + H of capon comb to 1/1000 cc. of preparation  $\theta$ SH*

(From Gallagher and Koch standard curve, J. Pharmacol. and Exptl. Therap., 1936, 55, 111)

Comb-growth L + H	$\theta$ SH 0.001 cc.	Comb-growth L + H	$\theta$ SH 0.001 cc.	Comb-growth L + H	$\theta$ SH 0.001 cc.	Comb-growth L + H	$\theta$ SH 0.001 cc.
mm.		mm.		mm.		mm.	
0.5	0.30	3.5	4.30	6.5	10.81	9.5	19.55
0.6	0.38	3.6	4.51	6.6	11.08	9.6	19.94
0.7	0.45	3.7	4.70	6.7	11.30	9.7	20.30
0.8	0.51	3.8	4.91	6.8	11.55	9.8	20.70
0.9	0.60	3.9	5.11	6.9	11.80	9.9	21.08
1.0	0.67	4.0	5.31	7.0	12.00	10.0	21.48
1.1	0.75	4.1	5.54	7.1	12.28	10.1	21.90
1.2	0.85	4.2	5.75	7.2	12.53	10.2	22.30
1.3	0.95	4.3	5.95	7.3	12.80	10.3	22.75
1.4	1.05	4.4	6.15	7.4	13.05	10.4	23.20
1.5	1.15	4.5	6.40	7.5	13.33	10.5	23.65
1.6	1.27	4.6	6.60	7.6	13.60	10.6	24.12
1.7	1.40	4.7	6.80	7.7	13.88	10.7	24.60
1.8	1.51	4.8	7.00	7.8	14.14	10.8	25.10
1.9	1.61	4.9	7.22	7.9	14.41	10.9	25.64
2.0	1.74	5.0	7.45	8.0	14.70	11.0	26.15
2.1	1.87	5.1	7.78	8.1	15.00	11.1	26.68
2.2	2.01	5.2	7.90	8.2	15.30	11.2	27.25
2.3	2.20	5.3	8.10	8.3	15.60	11.3	27.81
2.4	2.31	5.4	8.31	8.4	15.90	11.4	28.40
2.5	2.49	5.5	8.54	8.5	16.21	11.5	29.02
2.6	2.64	5.6	8.76	8.6	16.54	11.6	29.68
2.7	2.80	5.7	8.98	8.7	16.85	11.7	30.35
2.8	3.00	5.8	9.20	8.8	17.18	11.8	31.05
2.9	3.15	5.9	9.41	8.9	17.50	11.9	31.75
3.0	3.35	6.0	9.68	9.0	17.80		
3.1	3.54	6.1	9.89	9.1	18.18		
3.2	3.74	6.2	10.11	9.2	18.50		
3.3	3.95	6.3	10.37	9.3	18.85		
3.4	4.11	6.4	10.60	9.4	19.20		

*Example:* Suppose the standard preparation containing 1 international unit of male hormone (the equivalent of 100 gamma androsterone) per cc. gave an L + H value of 4.7 and your unknown, after correction to the same average initial comb-length as your standard used in parallel, gave an L + H value of 4.0. Then, since the equivalents in terms of 0.001 cc.  $\theta$ SH are 6.8 and 5.31 respectively, it follows that the unknown contains 5.31/6.8 or 0.78 international male hormone unit per cc.

excretion of androgens is very striking, ranging from 13 to 69 international units per day. In only one of the men, Subject 1, is there a suggestion of a cycle. In spite of these irregularities, the average daily excretion for the four men is remarkably constant, ranging from 38 to 41 international units. It should be noted that these values were obtained after 2 hours' acid hydrolysis of the urine at boiling temperature. Undoubtedly, all these values would have been appreciably higher by our latest modification of hydrolysis

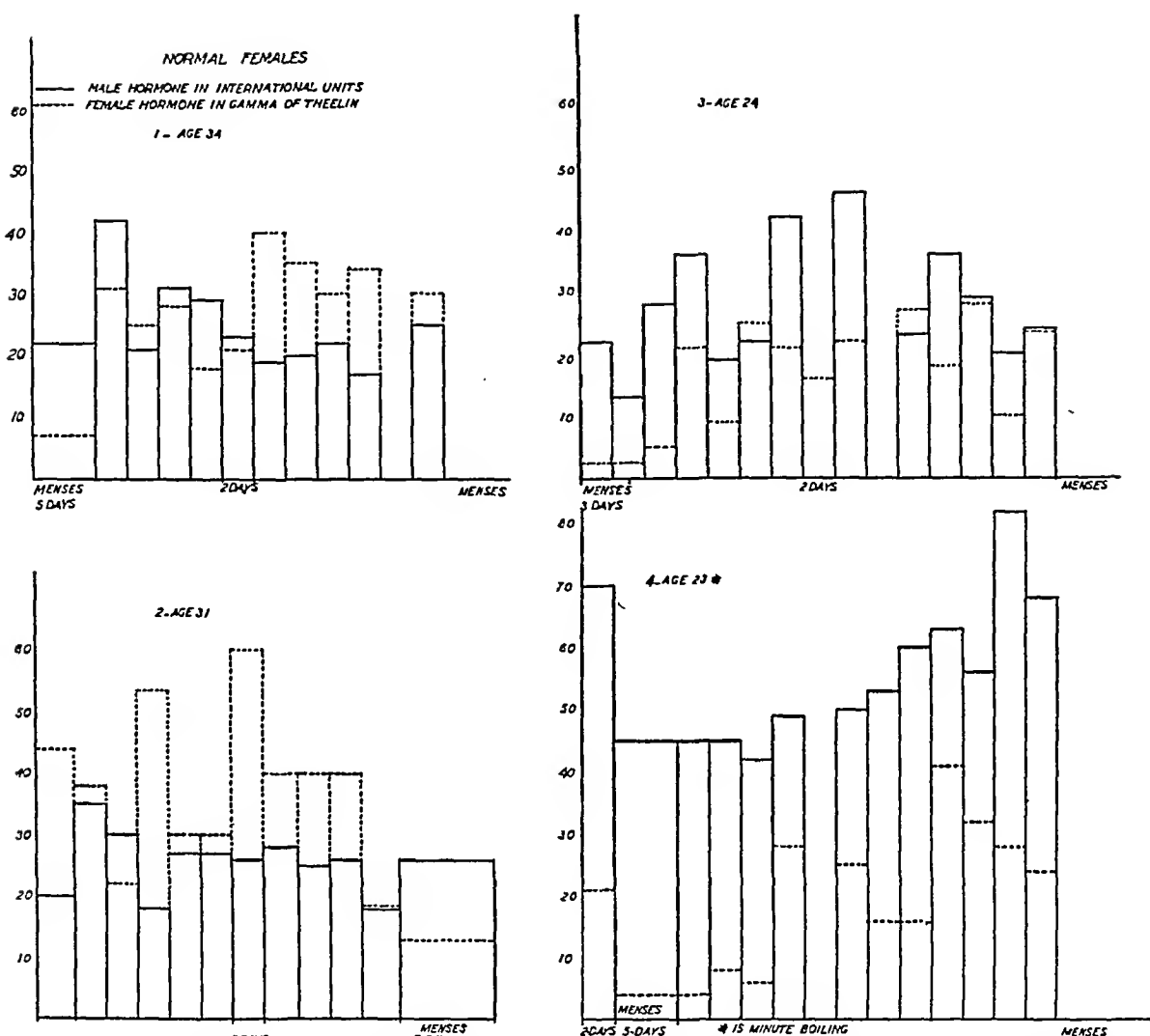


FIG. 3. THE EXCRETION OF ANDROGENIC AND ESTROGENIC SUBSTANCES IN THE URINE OF FOUR NORMAL WOMEN THROUGHOUT THE MENSTRUAL CYCLE

nitely established that hormone concentrates from testis tissue contain estrogenic material, the evidence for androgenic material in ovarian extracts is not so well established. Nevertheless, there is considerable evidence that comb-growth in the hen or pullet is due to an androgenic substance and that this probably comes from the ovarian medulla (19). Pure estrogenic substances do not cause comb-growth in the hen but pure androgens do. It is also possible that a number of androgenic and estrogenic materials may be formed in the metabolism of hypothetical common precursors for androgens and estrogens. This may in-

volve not only the normal or abnormal processes in the gonads, but also normal or pathological metabolism of sterols or other intermediates in the suprarenals and liver. That the suprarenal may be involved is suggested by the findings of Frank (20) and the authors (21) as well as by the oxidative degradation studies on one of the crystalline compounds isolated from the adrenal cortex (22, 23). To what extent our food contains sex hormones or precursors easily metabolized to active waste products remains to be seen. However, this source is not a very significant one directly, because castrates excrete only very small



TABLE II

*The daily urinary excretion of androgenic and estrogenic activities by four normal men\**

(The urine of these subjects was boiled for 2 hours before extraction)

Subject 1, age 27 years				Subject 2, age 26 years				Subject 3, age 30 years				Subject 4, age 35 years			
Days	An	Es	An Es	Days	An	Es	An Es	Days	An	Es	An Es	Days	An	Es	An Es
3	25	10	3	3	40	5	8	3	30	12	3	2	25	8	3
3	39	8	5	3	28	10	3	3	37	10	4	3	69	16	4
3	50	4	12	3	27	2	14	3	45	10	5	3	25	5	5
3	34	10	3	3	37	18	2	3	52	26	2	3	38	13	3
3	38	8	5	3	32	2	16	3	42	10	4	3	23	16	2
3	24	8	3	3	65	3	22	3	24	4	0	3	58	11	5
3	33	5	7	3	20	2	10	3	70	14	6	3	65	11	6
3	37	5	8	3	41	20	2	3	49	10	5	3	42	10	4
3	45	10	5	3	53	3	18	4	41	11	4	3	41	16	3
3	48	5	10	3	36	2	18	4	36	2	18	3	31	10	3
3	63	18	4	3	52	18	3	3	44	2	22	4	30	21	2
3	38	10	4	3	lost			3	40	20	1	3	34	10	3
3	34	10	3	3	46	18	3	3	lost						
3	29	9	3	3	62	8	8	3	13	14	1				
Average values	38	9	4.2		41	9	4.6		41	10	4.1		40	12	3.3

\* "An" designates international androgenic units per day. "Es" designates estrogenic activity as gamma of theelin per day.

basis of the 15-minute boiling process, these ratios are raised to range from 5.5 to 7.6 (Table IV).

*Women's urine.* The results on the four women are given in Figure 3 and Table III. The urines from Subjects 1, 2 and 3 were hydrolyzed by the 2-hour process, but in the case of Subject 4 the more reliable 15-minute boiling procedure was used. The increase in yield of androgens by the 15-minute process in Subject 4 as compared with the longer period of hydrolysis in Subjects 1, 2 and 3 is very striking and is confirmed by control experiments on many other studies on women's urine. In Subjects 1, 2 and 3, the androgenic units excreted per day range from 13 to 46 and again the same range holds for one individual. In Subject 4 the excretion is more constant, ranging from 42 to 85. The average values for the women are again very constant if calculated on the same basis (Table IV). They are, however, distinctly lower than the averages for men. A more extended series will be necessary for the perfectly satisfactory establishment of this sexual difference.

The daily excretion of estrogenic material expressed as theelin in the women ranges from 4 to 60 gamma. The average daily excretion on the four subjects varied from 18 to 27 gamma of theelin. Figure 2 shows that, although our estro-

genic assays may not have been as accurate as desired, there is a suggestion of two peaks during the cycle, one at 7 to 15 days after the onset of menstruation, and one at 6 to 12 days before the onset of the next menstrual period. In each case the excretion rate is lowest during menstruation. Our findings thus confirm the results reported by Gustavson and Green (13) and Frank (12). The significance of the peaks in the excretion of estrogens in women cannot be fully analyzed at the present time because of our incomplete knowledge on the changes in concentration of estrogens in blood and the relation thereof to ovarian-follicle and corpus-luteum formation and degeneration.

In women as in men the rates of excretion of androgenic and estrogenic substances in the urine bear no relation to each other. From Tables III and IV it is seen that the An/Es ratio is lower in women than in men. By the 2-hour boiling procedure this ratio ranges from 0.3 to 7, whereas in men it varied from 1 to 22. The average values of the ratio for these normal women ranges from 1.2 to 2.8 and 0.72 to 1.7 for the 15-minute and 2-hour boiling procedures respectively.

The source of these urinary hormones is, of course, of interest when we consider that both sexes excrete both types. Although it is defi-

TABLE III

*The daily urinary excretion of androgenic and estrogenic activities by four normal women\**

(The urine of Subject 4 was hydrolyzed for 15 minutes, the others for 2 hours)

Subject 1, age 34 years				Subject 2, age 31 years				Subject 3, age 24 years				Subject 4, age 23 years			
Days	An	Es	An Es	Days	An	Es	An Es	Days	An	Es	An Es	Days	An	Es	An Es
5	22	7	3	3	20	44	0.5	2	22	5	4	2	70	21	3
2	42	31	1	2	35	38	1.0	2	13	5	3	5	45	4	11
2	21	25	1	2	30	22	1	2	28	10	3	2	45	8	6
2	31	28	1	2	18	53	0.5	2	36	21	2	2	45	8	6
2	20	18	2	2	27	30	1.0	2	19	9	2	2	42	11	4
2	23	21	1	2	27	30	1.0	2	22	25	1	2	49	28	2
2	19	40	0.5	2	26	60	0.5	2	42	21	2	2	lost		
2	20	35	0.5	2	28	40	0.5	2	16	16	1	2	50	25	2
2	22	30	0.5	2	25	40	0.5	2	46	22	2	2	53	16	3
2	17	34	0.5	2	26	40	0.5	2	lost			2	60	16	4
2	lost			2	18	18	1	2	23	27	1	2	63	41	2
2	25	30	1.0	7	26	13	2	2	36	18	2	2	56	32	2
								2	29	28	1	2	85	28	3
								2	20	10	2	2	68	24	3
								2	24	22	1				
Average values	25	27	0.93		26	36	0.72		28	18	1.6		56	20	2.8

\* "An" designates international androgenic units per day. "Es" designates estrogenic activity as gamma of theelin per day.

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TABLE IV

*A summary of the average values from Tables II and III\**

Men						Women					
Subject	An <sup>15</sup>	An <sup>120</sup>	Es	An <sup>15</sup> Es	An <sup>120</sup> Es	Subject	An <sup>15</sup>	An <sup>120</sup>	Es	An <sup>15</sup> Es	An <sup>120</sup> Es
1	63†	38	9	7.0	4.2	1	42†	25	27	1.6	0.9
2	68†	41	9	7.6	4.6	2	43†	26	36	1.2	0.7
3	68†	41	10	6.8	4.1	3	47†	28	18	2.5	1.5
4	66†	40	12	5.5	3.3	4	56	34†	20	2.8	1.7
Average				6.9	4.1					2.0	1.2

\* "An<sup>15</sup>" designates the international androgenic units per day by the 15-minute boiling procedure.

"An<sup>120</sup>" the same by the 2-hour boiling-procedure.

"Es" designates the estrogenic activity as gamma of theelin per day.

† These values are calculated on the assumption that the yield of androgenic activity is increased by 66 per cent over the 2-hour hydrolysis if boiled 15 minutes instead.

amounts of these hormones. Even if foods did contain small amounts of these substances, their incomplete absorption probably would eliminate this factor as a significant source.

The fluctuations in the urinary excretion of these hormones in normal men and women should serve as a warning to all of us not to place too much emphasis on the assays of one or two 24-hour samples from one individual.

#### SUMMARY

1. A quantitative method for the extraction of androgenic and estrogenic materials from urine is given in detail.

2. This method has been applied on the urines of four normal men and four normal women over a continuous period of 39 to 45 days for the men and over a complete menstrual cycle for the women.

3. There are marked fluctuations in the daily urinary excretion of androgens and estrogens in normal men and women.

4. There is no definite evidence of a monthly cycle in the excretion of either androgens or estrogens in normal men. In normal women the excretion of estrogens is characteristically low during the menstrual flow and rises during the intermenstruum with a double peak in certain instances.

5. The average daily excretions of androgens obtained by our best methods of hydrolysis and extraction are 63 to 68 units for the men studied

and 42 to 56 units for the women studied, calculated as international androgen units. Our women, therefore, excreted two-thirds as much androgenic material as our men. An extended series is desirable to establish further such a difference between the sexes.

6. The average daily excretions of estrogens, calculated as gammas of theelin, are 9 to 12 gammas for men and 18 to 36 gammas for women.

7. The rates of excretion of androgenic and estrogenic substances do not seem to bear any relation to each other in either sex.

8. On the basis of the best known methods of hydrolysis and extraction, the ratios obtained by dividing the international androgenic units excreted per day by the gammas of theelin excreted per day vary considerably for the same individual. The average values for the eight subjects ranged from 5.5 to 7.6 for men and 1.2 to 2.8 for women.

#### ADDENDUM

Since this paper was written an important contribution by Dingemans, Borchardt and Laqueur (24) has appeared in which the usual male hormone values for the urine of normal men up to the age of 40 are given as 40 to 50 international units per liter. Aged men excreted from 5 to 40 units per liter. These workers differ from us in obtaining no essential difference between men and women. A one and one-half month study of one normal man gives no evidence of cycle, in agreement with our own observations. In one normal woman studied through a cycle there was a slight rise in the excretion of androgenic substances post-menstrually. Small amounts of androgenic material were found consistently in the urine of children.

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# THE URINARY EXCRETION OF ANDROGENIC AND ESTROGENIC SUBSTANCES IN CERTAIN ENDOCRINE STATES. STUDIES IN HYPOGONADISM, GYNecomastia AND VIRILISM<sup>1</sup>

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The comparison of the excretion of male and female hormone-like substances with the clinical expressions of disease may be expected to teach us the extent to which studies of the urine reveal the changing functions of the gonads and may help to illuminate the difficult pathological physiology of such obscure processes as virilism and gynecomastia. As a step in this direction we are presenting data on castration in the male, eunuchoidism, hypopituitarism, bilateral cryptorchidism without alteration in secondary sex characters, gynecomastia, precocious puberty and virilism.

The assays were conducted according to the technique of Gallagher, Koch and Dorfman (1) as outlined in the preceding paper dealing with the normal (2). In all cases complete urine collections were secured through a number of days. During the bulk of the work the acidified urine was boiled for two hours to insure the maximum yield of estrogenic material. When it was learned that briefer boiling gives measurably higher values for male hormone than either boiling for two hours or none at all, this procedure was introduced in a few instances. The urine was then extracted with benzene in a continuous extractor, the benzene distilled off and the residue distributed between ether and 10 per cent aqueous sodium hydroxide. Ninety-five per cent of the estrogenic material passes into the alkali and none of the androgenic. The androgenic fraction was assayed on from four to seven capons according to the technique of Gallagher and Koch (3) and the estrogenic fraction on ten spayed adult female rats, according to the vaginal spread technique of D'Amour and Gustavson (4). As emphasized in the preceding paper, rigid comparisons were always made with standard preparations to permit control of the inevitable variations in the animal

colonies and the environment. The values for male hormone are expressed in the newly adopted international unit, each unit representing the activity equivalent to that of 0.1 mgm. of androsterone. The values for estrogenic material are expressed in  $\gamma$  of theelin (0.001 mgm. or 10 international estrogenic units).

It must not be supposed that our assays estimate single pure substances. Both androsterone and dehydroandrosterone have been shown to be constituents of the comb-growth stimulating mixture in urine, and both theelin (oestrone) and theelol (oestriol) are probably present. Quite possibly some of the many known relatives of these may in time be identified in urine. The suggestion of Deanesly and Parkes (5) that some of the estrogenic activity in male urine is due to dehydroandrosterone is not applicable to our data because this substance will not pass into the alkaline fraction on which the female assays are made. No attempts have been made in our studies to determine separately the estrogenic activity extractable from fresh urine and that larger amount extractable from hydrolyzed urine although useful information may well be secured in this way, as in Cohen and Marrian's studies of pregnancy. Our observations are of "total estrogens."

## I. HYPOGONADISM IN THE MALE

### A. Castration

The castrated individual provides the proper base line for all studies on the excretion of sex hormones. In the absence of the gonads the continued presence of such material in the urine must be attributed either to some other organ or to the food. McCullagh and Renshaw (6) found no comb-growth promoting material in the chloroform extracts of the urine of eleven eunuchs. Of these, seven were 50 years of age or more, an age group in which it has been claimed but not sub-

<sup>1</sup> These investigations were supported in part by a grant from the Rockefeller Foundation.



age 56. The patient was obese and effeminate and had large breasts. At the age of 19 he had supposedly been castrated because of infection following a bilateral operation for undescended testicles. A complete collection of urine amounting to 20.2 liters was secured.

Traces only of androgenic and estrogenic material were present in the urine (Table I). This is in accord with the view that the bulk of the hormone-like constituents of male urine are of gonadal origin and suggests that traces may come from elsewhere. These studies, however, must be extended as suitable material presents itself.

### B. Eunuchoidism—Hypopituitarism

Seven men studied represent that form of hypogonadism known as eunuchoidism since the early descriptions of Tandler and Grosz (31). While commonly recognized, the syndrome has received little attention in the American literature. It is characterized by hypoplasia of the genitalia and their accessories with impairment of the mechanism of ejaculation, retardation in development of the secondary sex characters, delay in closure of the epiphyses of the long bones, and absence of obvious constitutional disturbance. While presumably a wide variety of agents injuring the testes or the pituitary body may cause the syndrome, one usually cannot find clear indications of them. If defect in the pituitary is responsible, the gonadotropic properties must alone be involved. The most detailed pathological study available, that of Altmann (9), certainly has disclosed no considerable pituitary lesion, and the variety and inconsistency of the microscopic findings leaves one much in the air as to the rôle of this organ. Such attempts as we and others (10, 11) have made to find excessive prolactin in the urine have failed, but we hesitate to make too much of this as proof of primary responsibility of the hypophysis.

No studies have been made to our knowledge of the excretion of male and female hormones in this syndrome. A more detailed clinical study of these people will be reported subsequently.

*N. T. (Referred by Dr. I. Becker) (University of Chicago Clinics number 87890), age 26.* The patient was of normal height and weight with scant pubic and axillary hair, and only a slight fuzz on the upper lip. The laryngeal prominence was absent and the voice boy-

ish. The testes were small, measuring  $2.7 \times 1.7 \times 1.3$  cm., the epididymides small and the rudimentary prostate, barely detectable when first examined, was, two years later (January 1936), well defined but still very small. The penis was 4.7 cm. long. Erections occurred occasionally and small ejaculates of albuminous material have been procurable since August 1935. Creatinuria was demonstrable. Basal metabolism, glucose tolerance, visual fields, and x-rays of the sella turcica were normal. Numerous epiphyseal lines were open from three to five years beyond the slowest normal. The urine as reported by Dr. Z. Wallen-Lawrence did not contain the large amount of gonadotropic material present in that of many castrates.

Assays were performed on a composite specimen of 6,500 cc. collected between January 24 and May 1, 1934, on a 7-day specimen between September 5 and 12, 1934, on a complete 10-day specimen between October 6 and 15, 1934, and on a complete 7-day collection ending January 7, 1936.

*F. R. (University of Chicago Clinics number 113589), age 24.* Of normal height and weight with deficient axillary and pubic hair and a smooth face, the patient had a history both of serious head injury and of a dermatitis involving the scrotum. The laryngeal prominence was absent, and the voice somewhat high in pitch. The testes measured  $2.2 \times 1.4 \times 1.1$  cm., the epididymides were small and the prostate just apparent. The penis was 4.7 cm. long. Erections occurred occasionally, ejaculates never. Creatinuria was demonstrable. Basal metabolism, glucose tolerance, visual fields, and x-rays of the sella turcica were normal. Numerous epiphyseal lines were unfused two to six years beyond the slowest normal. The urine did not contain the large amount of gonadotropic material present in that of many castrates.

Two complete 7-day collections in November 1934 and one complete 7-day collection ending January 7, 1936, were assayed.

*N. D. (Referred by Dr. M. Kolaczros) (University of Chicago Clinics number 134245), age 29.* The patient was very thin and of normal height with scant pubic and axillary hair and a smooth face. The laryngeal prominence was absent, and the voice was somewhat high in pitch. The testes measured  $2.2 \times 1.5 \times 1.4$  cm., the epididymides were small, and the prostate practically imperceptible. The penis was 4.2 cm. long. Erections occurred but no ejaculations. Creatinuria has been observed. Basal metabolism, glucose tolerance, visual fields and x-rays of the sella turcica showed nothing unusual. Various epiphyseal lines were unfused from four to nine years beyond the slowest normal. His arms and legs were disproportionately long. The urine did not contain the large amount of gonadotropic material present in that of the castrate.

Complete 7-day urine samples during August 1935 and January 1936 were examined.

*H. K. (University of Chicago Clinics number 121217), age 23.* The patient was of normal height and somewhat

TABLE I  
The excretion of sex hormones in hypogonadism in the male

Patient	Age	Condition	Duration of collection	Duration of boiling	Number of cnpns	International androgen units per day		Theelin per day	Ratio androgens to $\gamma$ theelin	
						15 minutes	2 hours		15 minutes	2 hours
R. S.....	21	Castrate	18 days	120	4		1/liter	$\gamma$ 3/liter		0.3
J. W.....	56	Castrate	20.2 liters	120	4		3.5/liter	4.5/liter		0.8
N. T.....	26	Gynecomastia Eunuchoid	6.5 liters	120	4		17/liter	2/liter		8.5
	27		7 days	120	4		0/day	2/day		0.5
			10 days	120	4		9	2		4.5
	29		7 days	15	7	15	9*	1	15.0	9.0*
F. R.....	24	Eunuchoid	7 days	120	4		9	2		4.5
			7 days	120	4		11	2		5.2
	26		7 days	15	7	28	17*	1	28.0	17.0*
N. D.....	29	Eunuchoid	7 days	120	7		11			
			7 days	15	7	7	5*	1	7.0	5.0*
H. K.....	23	Eunuchoid	7 days	120	4		17	1		17.0
A. M.....	24	Cryptorchid	6 days	15	7	18	11*	2	9.0	5.5*
	20	Eunuchoid	3 days	15	7	12	7*	9	1.3	0.8*
D. V.....	31	Cryptorchid	3 days	15	7	21	13*	5	4.2	2.6*
	36	Eunuchoid	3 days	15	7	21	13*	1	21.0	13.0*
J. H.....		Cryptorchid				33	19*			
L. H.....	30	Hypopituitary	11 liters	120	4		4/liter	3/liter		1.3
E. H.....	26	Cryptorchid	7 days	120	4		31	1		31.0
			8 days	120	4		9	6		1.6
J. C.....	13	Cryptorchid	4 days	120	7		19	<5		3.6+
Average.....		7 Eunuchoids	3 to 10 days	15 to 120	4 to 7	20	12.9	1.8	13.8	7.6
Average.....		4 Normals	6 weeks	2 hours	7	67*	40.0	10.0	6.7	4.1

\* Calculated on the assumption that the 2-hour hydrolysis gives values 60 per cent of those secured by the 15-minute hydrolysis. This relationship may also be expressed by the statement that the 15-minute hydrolysis gives values 66 per cent above that of the 2-hour hydrolysis.

stantiated that the male hormone excretion is normally diminished. Eng (7) found 2 to 15 mouse units per liter of estrogenic material in the urine of three castrated women and 2 to 17 mouse units per liter in the urine of four castrated men. Five to 15 mouse units per day were excreted in the feces of the eunuchs. In one patient, nine days on a "folliculin"-free diet caused no decline in the excretion of estrogenic material which Eng therefore felt was of endogenous origin. Frank, Goldberger and Salmon (8) found that the urine of twelve women castrated surgically and 3 castrated by x-ray contained from 15 to 200 mouse units of estrogenic substances per liter. In two cases, 255 and 720 mouse units were excreted per month as compared to 1,500 in the normal. Dingemans, Borchardt and Laqueur (32) have recently reported 6 to 12 international androgen units per liter in the urine of three oophorectomized women.

We have been able to examine the urine of two castrated men. Unfortunately, neither case is beyond criticism, as one was 56, belonging to an insufficiently understood age group and the other had schizophrenia which may itself have had some influence. Furthermore, in the former, confirmation of the surgical procedure is now impossible and the gynecomastia present raises doubts concerning the completeness of the castration.

R. S. (Referred by Dr. J. M. Austin), negro, age 21. The patient, severely affected with dementia praecox of catatonic type, had been completely castrated by a fellow patient at the South Carolina State Hospital, Columbia, South Carolina, in January 1934. Practically the entire penis had been removed in the mutilation. The urine was collected with great difficulty through the generous efforts of Dr. James M. Austin, by an inlying catheter during eighteen of twenty-one days in December 1934. No physical consequence of the castration had been noted in twelve months.

J. W. (Referred by Dr. O. W. Thompson), negro,

### *C. Cryptorchidism without alteration in secondary sex characters*

Our data on this point are scant. It is of interest that Patient E. H., who had well-developed secondary sex characters, reached well into the normal range on one of two assays for androgenic material. The other patient, J. C., is 13 and while there are some signs of puberty, it will be several years before the degree of retardation in the development of secondary sex characters can be accurately judged (Table I).

*E. H. (Referred by Dr. W. M. Brunet) (University of Chicago Clinics number 118497), age 26.* The patient was bilaterally cryptorchid, the left testis being in the inguinal canal and the right probably at the ring, where some testicular sensation could be secured by pressure. His ejaculate contained no sperm but his genitalia and secondary sex characters were well developed.

Complete urine collections for one 7-day and one 8-day period were made in January 1935.

*J. C. (Referred by Dr. H. T. Ricketts) (University of Chicago Clinics number 137064), age 13.* The patient was obese with impalpable testes, showed some pubic hair and a small well-defined prostate. Roentgenogram of the sella turcica showed no pathology. His basal metabolic rate was  $-28$ .

Two 48-hour urine specimens were obtained in September 1935.

### II. PRECOCIOUS PUBERTY

We have been able, through the efforts of Dr. Thomas Myers of St. Paul, to examine the urine of one patient, a boy of five, with precocious puberty of unknown etiology. Unhappily, we could

pounds and was strong and muscular. Pubic hair and rapid growth of the genitalia had been observed during the preceding year. His voice was bass, and acne had been present on his face for one and a half years. X-rays of the carpus showed all eight bones present and well developed as at 12. The skull was not definitely abnormal. Three basal metabolism tests showed  $+36$ ,  $+40$  and  $+57$ , but clinical signs of hyperthyroidism appeared to be absent. Mentally he was sound, and no evidences of unusual libido were present.

A complete urine collection for seven days was secured in the winter of 1935.

### III. GYNECOMASTIA

The appearance of feminine breast tissue in men is rare and somewhat difficult to explain. Lewis and Geschickter (12), who have examined the tissues of ninety-five instances of gynecomastia, find consistently a proliferation of the ducts and periductile connective tissue, with no alveolus formation, a picture similar to that in virginal breast hypertrophy. As such a development has been produced by giving estrin to male monkeys and is in accord with the widely known effects of estrin in other species (13), it is most natural to suppose this feminizing material to be the active agent. Testosterone benzoate, however, was also shown to stimulate the tubuloacinar system of the breast of the rat to some extent (14), and androstane-diol, a synthetic relative of the male hormone, not appearing as far as is known in the animal body, has a powerful mammotropic effect in the rat (15). Unexpected difficulties with the theory of hyperfunction of the testis, the presumable source of either the estrogenic substances or testosterone, are encountered, however, when the clinical associations of gynecomastia are reviewed, for testicular atrophy is common (16). While such secondary sex characters as hair-growth may be deficient they are usually normal in these people. Whether interstitial cell hypertrophy, which is known to accompany testicular atrophy frequently, will be found often enough to provide a source for the active agent, is still uncertain. When chorioepithelioma produces gynecomastia, the proliferating decidua-like tissue may readily be conceived a source of augmented estrin secretion, but even here we cannot be too casual. Hamburger (10) in a most careful study found gynecomastia when the amount of gonadotropic material in the urine was the least and in a patient in which the second testis had been destroyed.

TABLE II  
*The excretion of sex hormones in gynecomastia and precocious puberty*

Patient	Age	Condition	Duration of collection	Duration of boiling	Number of capons	International androgen units/day	Thelin per day	Ratio androgens to $\gamma$ theelin
	years		days	hours			$\gamma$	
K. L. ....	16	Gynecomastia	6	2	7	37	15	2.5
J. E. ....	24	Gynecomastia	3	7	7	00	12	0.1—
T. W. ....	15	Gynecomastia	7	2	6	8	5	1.6
Boy .....	5	Precocious puberty	7	2	6	00		

demonstrate no male hormone, a finding of which we are skeptical and which requires confirmation. It is conceivable that the high basal metabolism may have modified the results (Table II).

*This boy, age 5, was 48½ inches tall, weighed 58*



heavy, with little pubic and axillary hair and a smooth face. The laryngeal prominence was not present, but the voice was normal. The testes were not present and expert surgical intervention disclosed them too high to be effectually drawn down. The prostate could not be felt. The penis was 3.5 cm. long. Erections occurred but no ejaculations. Glucose tolerance showed a curve of mild diabetes; basal metabolism, visual fields and x-rays of the sella turcica were normal. There was evidence of delay in epiphyseal fusion of as much as five years.

A 7-day collection during August 1935, and a 6-day collection during May 1936, were assayed.

*A. M. (Referred by Dr. Henry Schmitz) (University of Chicago Clinics number 152001), age 20.* The patient was of normal height but quite obese with scant axillary and pubic hair and a small amount of terminal hair on the upper lip which had remained as at present for five years. The laryngeal prominence was slight and the voice boyish. The testes could not be felt, nor could the prostate. The penis was 3.5 cm. long. Erections but no ejaculates occurred. Basal metabolism, glucose tolerance, visual fields and x-rays of the sella turcica were normal. There was little significant delay in epiphyseal fusion.

Two 3-day collections in June 1936 were assayed.

*D. V. (Referred by Dr. Paul Bucy) (University of Chicago Clinics number 41111), age 31.* The patient was of normal height and weight with a small amount of axillary and pubic hair and smooth upper lip. A small laryngeal prominence was apparent and his voice approached normal. The testes measured  $3.5 \times 2.0 \times 1.7$  cm., the prostate was small but definite. The penis varied from 3.8 to 6.2 cm. long. Erections but no ejaculations occurred. Basal metabolism, glucose tolerance and x-rays of the sella turcica were normal. Bilateral optic atrophy of moderate grade had been present for years, and had not progressed. The fusion of several epiphyses was delayed seven to eight years.

A 3-day collection in July 1936 was assayed.

*J. H. (Referred by Drs. Carl Moore and C. B. Huggins) (University of Chicago Clinics number 160510), age 36.* The patient was of normal height and weight with a small amount of axillary and pubic hair and only a little fine fuzz on the upper lip. The laryngeal prominence was small, and his voice was normal. Neither testis was palpable. The penis was 4.1 cm. long. Erections but no ejaculations occurred. Basal metabolism, glucose tolerance, visual fields and sella turcica roentgenogram were normal. Several unfused epiphyseal lines were present.

A 3-day collection in October 1936 was assayed.

We have also been able to examine the urine of one patient in which the hypogonadism was proved to be due to a chromophobe tumor of the pituitary body.

*L. H. (Referred by Drs. Percival Bailey and Paul Bucy) (University of Chicago Clinics number 87850), age 30.* The patient suffered from weakness, decline in

vision, loss of body hair and impotence. There was a globular enlargement of the sella turcica, with a depression of its floor on x-ray. Visual fields showed a right temporal hemianopsia. His basal metabolism was —40 per cent and below on several occasions and his blood pressure was not infrequently as low as 86/54. X-ray therapy to the pituitary region had been given before the urine collection was made. He has since shown improvement in his general condition, his vision has not further declined, but he is still impotent.

A complete 11-liter urine collection was made in May 1934 and assayed.

Table I presents the data on these instances of hypogonadism. It will be recalled from the preceding paper that the four normal young men studied continuously for six weeks averaged in the neighborhood of 40 international androgen units of male hormone per day each, with a range of 13 to 79, seven capons being used for each assay and the urine being boiled for two hours before extraction. If we allow for the destruction of 40 per cent of the androgenic material between the 15 minute hydrolysis and the two hour hydrolysis, as described in the preceding paper, this average normal value becomes 67 international units per day, and the range 22 to 132. In contrast with this the seven-capon assays on the seven eunuchoids give an average value of 20 units of male hormone, with a range of 7 to 33, by the 15 minute hydrolysis. The four capon assays with the two hour boiling on the eunuchoids give good corroboration. It should be noted that there is some overlapping of the normal and the hypogonad. This is not surprising when we recall that all of our eunuchoids have developed such secondary sex characters as axillary and pubic hair to the extent reached by the fourteen or fifteen year old boy, and thus present some clinical evidences of testicular activity. On the average, our hypogonads excrete about a third of the normal amount of male hormone.

With the exception of one assay on Patient A. M. the excretion of estrogenic material is reduced to traces in the eunuchoid, and while this level is reached on occasion by the normal, the average normal value is 10  $\gamma$  per day. The values for both male and female hormones in the patient with hypopituitarism (L. H.) are low.

pounds and was strong and muscular. Pubic hair and rapid growth of the genitalia had been observed during the preceding year. His voice was bass, and acne had been present on his face for one and a half years. X-rays of the carpus showed all eight bones present and well developed as at 12. The skull was not definitely abnormal. Three basal metabolism tests showed +36, +40 and +57, but clinical signs of hyperthyroidism appeared to be absent. Mentally he was sound, and no evidences of unusual libido were present. A complete urine collection for seven days was secured in the winter of 1935.

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J. B. ....	16	Gynecomastia	3	2	6	00	12
T. W. ....	16	Gynecomastia	7	2	6	00	0.1—
Boy.....	5	Precocious puberty	7	2	2	00	1.6

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*A. M. (Referred by Dr. Henry Schmitz) (University of Chicago Clinics number 152001), age 20.* The patient was of normal height but quite obese with scant axillary and pubic hair and a small amount of terminal hair on the upper lip which had remained as at present for five years. The laryngeal prominence was slight and the voice boyish. The testes could not be felt, nor could the prostate. The penis was 3.5 cm. long. Erections but no ejaculates occurred. Basal metabolism, glucose tolerance, visual fields and x-rays of the sella turcica were normal. There was little significant delay in epiphyseal fusion.

Two 3-day collections in June 1936 were assayed.

*D. V. (Referred by Dr. Paul Bucy) (University of Chicago Clinics number 41111), age 31.* The patient was of normal height and weight with a small amount of axillary and pubic hair and smooth upper lip. A small laryngeal prominence was apparent and his voice approached normal. The testes measured  $3.5 \times 2.0 \times 1.7$  cm., the prostate was small but definite. The penis varied from 3.8 to 6.2 cm. long. Erections but no ejaculations occurred. Basal metabolism, glucose tolerance and x-rays of the sella turcica were normal. Bilateral optic atrophy of moderate grade had been present for years, and had not progressed. The fusion of several epiphyses was delayed seven to eight years.

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We have also been able to examine the urine of one patient in which the hypogonadism was proved to be due to a chromophobe tumor of the pituitary body.

*L. H. (Referred by Drs. Percival Bailey and Paul Bucy) (University of Chicago Clinics number 87850), age 30.* The patient suffered from weakness, decline in

vision, loss of body hair and impotence. There was a globular enlargement of the sella turcica, with a depression of its floor on x-ray. Visual fields showed a right temporal hemianopsia. His basal metabolism was -40 per cent and below on several occasions and his blood pressure was not infrequently as low as 86/54. X-ray therapy to the pituitary region had been given before the urine collection was made. He has since shown improvement in his general condition, his vision has not further declined, but he is still impotent.

A complete 11-liter urine collection was made in May 1934 and assayed.

Table I presents the data on these instances of hypogonadism. It will be recalled from the preceding paper that the four normal young men studied continuously for six weeks averaged in the neighborhood of 40 international androgen units of male hormone per day each, with a range of 13 to 79, seven capons being used for each assay and the urine being boiled for two hours before extraction. If we allow for the destruction of 40 per cent of the androgenic material between the 15 minute hydrolysis and the two hour hydrolysis, as described in the preceding paper, this average normal value becomes 67 international units per day, and the range 22 to 132. In contrast with this the seven-capon assays on the seven eunuchoids give an average value of 20 units of male hormone, with a range of 7 to 33, by the 15 minute hydrolysis. The four capon assays with the two hour boiling on the eunuchoids give good corroboration. It should be noted that there is some overlapping of the normal and the hypogonad. This is not surprising when we recall that all of our eunuchoids have developed such secondary sex characters as axillary and pubic hair to the extent reached by the fourteen or fifteen year old boy, and thus present some clinical evidences of testicular activity. On the average, our hypogonads excrete about a third of the normal amount of male hormone.

With the exception of one assay on Patient A. M. the excretion of estrogenic material is reduced to traces in the eunuchoid, and while this level is reached on occasion by the normal, the average normal value is 10  $\gamma$  per day. The values for both male and female hormones in the patient with hypopituitarism (L. H.) are low.

heavy, with little pubic and axillary hair and a smooth face. The laryngeal prominence was not present, but the voice was normal. The testes were not present and expert surgical intervention disclosed them too high to be effectually drawn down. The prostate could not be felt. The penis was 3.5 cm. long. Erections occurred but no ejaculations. Glucose tolerance showed a curve of mild diabetes; basal metabolism, visual fields and x-rays of the sella turcica were normal. There was evidence of delay in epiphyseal fusion of as much as five years.

A 7-day collection during August 1935, and a 6-day collection during May 1936, were assayed.

*A. M. (Referred by Dr. Henry Schmitz) (University of Chicago Clinics number 152001), age 20.* The patient was of normal height but quite obese with scant axillary and pubic hair and a small amount of terminal hair on the upper lip which had remained as at present for five years. The laryngeal prominence was slight and the voice boyish. The testes could not be felt, nor could the prostate. The penis was 3.5 cm. long. Erections but no ejaculates occurred. Basal metabolism, glucose tolerance, visual fields and x-rays of the sella turcica were normal. There was little significant delay in epiphyseal fusion.

Two 3-day collections in June 1936 were assayed.

*D. V. (Referred by Dr. Paul Bucy) (University of Chicago Clinics number 4111), age 31.* The patient was of normal height and weight with a small amount of axillary and pubic hair and smooth upper lip. A small laryngeal prominence was apparent and his voice approached normal. The testes measured  $3.5 \times 2.0 \times 1.7$  cm., the prostate was small but definite. The penis varied from 3.8 to 6.2 cm. long. Erections but no ejaculations occurred. Basal metabolism, glucose tolerance and x-rays of the sella turcica were normal. Bilateral optic atrophy of moderate grade had been present for years, and had not progressed. The fusion of several epiphyses was delayed seven to eight years.

A 3-day collection in July 1936 was assayed.

*J. H. (Referred by Drs. Carl Moore and C. B. Huggins) (University of Chicago Clinics number 160510), age 36.* The patient was of normal height and weight with a small amount of axillary and pubic hair and only a little fine fuzz on the upper lip. The laryngeal prominence was small, and his voice was normal. Neither testis was palpable. The penis was 4.1 cm. long. Erections but no ejaculations occurred. Basal metabolism, glucose tolerance, visual fields and sella turcica roentgenogram were normal. Several unfused epiphyseal lines were present.

A 3-day collection in October 1936 was assayed.

We have also been able to examine the urine of one patient in which the hypogonadism was proved to be due to a chromophobe tumor of the pituitary body.

*L. H. (Referred by Drs. Percival Bailey and Paul Bucy) (University of Chicago Clinics number 87850), age 30.* The patient suffered from weakness, decline in

vision, loss of body hair and impotence. There was a globular enlargement of the sella turcica, with a depression of its floor on x-ray. Visual fields showed a right temporal hemianopsia. His basal metabolism was  $-40$  per cent and below on several occasions and his blood pressure was not infrequently as low as 86/54. X-ray therapy to the pituitary region had been given before the urine collection was made. He has since shown improvement in his general condition, his vision has not further declined, but he is still impotent.

A complete 11-liter urine collection was made in May 1934 and assayed.

Table I presents the data on these instances of hypogonadism. It will be recalled from the preceding paper that the four normal young men studied continuously for six weeks averaged in the neighborhood of 40 international androgen units of male hormone per day each, with a range of 13 to 79, seven capons being used for each assay and the urine being boiled for two hours before extraction. If we allow for the destruction of 40 per cent of the androgenic material between the 15 minute hydrolysis and the two hour hydrolysis, as described in the preceding paper, this average normal value becomes 67 international units per day, and the range 22 to 132. In contrast with this the seven-capon assays on the seven eunuchoids give an average value of 20 units of male hormone, with a range of 7 to 33, by the 15 minute hydrolysis. The four capon assays with the two hour boiling on the eunuchoids give good corroboration. It should be noted that there is some overlapping of the normal and the hypogonad. This is not surprising when we recall that all of our eunuchoids have developed such secondary sex characters as axillary and pubic hair to the extent reached by the fourteen or fifteen year old boy, and thus present some clinical evidences of testicular activity. On the average, our hypogonads excrete about a third of the normal amount of male hormone.

With the exception of one assay on Patient A. M. the excretion of estrogenic material is reduced to traces in the eunuchoid, and while this level is reached on occasion by the normal, the average normal value is  $10 \gamma$  per day. The values for both male and female hormones in the patient with hypopituitarism (L. H.) are low.

### C. Cryptorchidism without alteration in secondary sex characters

Our data on this point are scant. It is of interest that Patient E. H., who had well-developed secondary sex characters, reached well into the normal range on one of two assays for androgenic material. The other patient, J. C., is 13 and while there are some signs of puberty, it will be several years before the degree of retardation in the development of secondary sex characters can be accurately judged (Table I).

*E. H. (Referred by Dr. W. M. Brunet) (University of Chicago Clinics number 118497), age 26.* The patient was bilaterally cryptorchid, the left testis being in the inguinal canal and the right probably at the ring, where some testicular sensation could be secured by pressure. His ejaculate contained no sperm but his genitalia and secondary sex characters were well developed.

Complete urine collections for one 7-day and one 8-day period were made in January 1935.

*J. C. (Referred by Dr. H. T. Ricketts) (University of Chicago Clinics number 137064), age 13.* The patient was obese with impalpable testes, showed some pubic hair and a small well-defined prostate. Roentgenogram of the sella turcica showed no pathology. His basal metabolic rate was —28.

Two 48-hour urine specimens were obtained in September 1935.

## II. PRECOCIOUS PUBERTY

We have been able, through the efforts of Dr. Thomas Myers of St. Paul, to examine the urine of one patient, a boy of five, with precocious puberty of unknown etiology. Unhappily, we could

TABLE II  
*The excretion of sex hormones in gynecomastia and precocious puberty*

Patient	Age	Condition	Duration of collection	Duration of boiling	Number of capons	International androgen units/day	Theelin per day	Ratio androgens to $\gamma$ theelin
			days	hours				
K. L. ....	16	Gynecomastia	6	2	7	37	15	2.5
J. B. ....	24	Gynecomastia	3	2	7	00	12	0.1—
T. W. ....	15	Gynecomastia	7	2	6	8	5	1.6
Boy .....	5	Precocious puberty	7	2	6	00		

demonstrate no male hormone, a finding of which we are skeptical and which requires confirmation. It is conceivable that the high basal metabolism may have modified the results (Table II).

*This boy, age 5, was 48½ inches tall, weighed 58*

pounds and was strong and muscular. Pubic hair and rapid growth of the genitalia had been observed during the preceding year. His voice was bass, and acne had been present on his face for one and a half years. X-rays of the carpus showed all eight bones present and well developed as at 12. The skull was not definitely abnormal. Three basal metabolism tests showed +36, +40 and +57, but clinical signs of hyperthyroidism appeared to be absent. Mentally he was sound, and no evidences of unusual libido were present.

A complete urine collection for seven days was secured in the winter of 1935.

## III. GYNECOMASTIA

The appearance of feminine breast tissue in men is rare and somewhat difficult to explain. Lewis and Geschickter (12), who have examined the tissues of ninety-five instances of gynecomastia, find consistently a proliferation of the ducts and periductile connective tissue, with no alveolus formation, a picture similar to that in virginal breast hypertrophy. As such a development has been produced by giving estrin to male monkeys and is in accord with the widely known effects of estrin in other species (13), it is most natural to suppose this feminizing material to be the active agent. Testosterone benzoate, however, was also shown to stimulate the tubuloacinar system of the breast of the rat to some extent (14), and androstane-diol, a synthetic relative of the male hormone, not appearing as far as is known in the animal body, has a powerful mammotropic effect in the rat (15). Unexpected difficulties with the theory of hyperfunction of the testis, the presumable source of either the estrogenic substances or testosterone, are encountered, however, when the clinical associations of gynecomastia are reviewed, for testicular atrophy is common (16). While such secondary sex characters as hair-growth may be deficient they are usually normal in these people. Whether interstitial cell hypertrophy, which is known to accompany testicular atrophy frequently, will be found often enough to provide a source for the active agent, is still uncertain. When chorioepithelioma produces gynecomastia, the proliferating decidua-like tissue may readily be conceived a source of augmented estrin secretion, but even here we cannot be too casual. Hamburger (10) in a most careful study found gynecomastia when the amount of gonadotropic material in the urine was the least and in a patient in which the second testis had been destroyed.

The data on the excretion of hormones in gynecomastia is scant. Heidrich, Fels and Mathias (17) found 250 mouse units per liter in a man with teratoma of the testicle. Hamburger (10) detected estrogenic activity in the urine of one such patient and not in that of another.

We have been able to examine the urine of four patients with bilateral gynecomastia. One, a young boy, in whom the enlargement did not greatly exceed that which Jung and Shafton (18) have shown to be common in adolescent boys, also had a mild hyperthyroidism which may well have accentuated the process (19). One patient had hypoplasia of the testes, one an enlarged painful testicle following mumps orchitis, and one, a cryptorchid (J. W., summarized as a castrate), had been subjected, supposedly, to a bilateral orchidectomy.

*K. L. (Referred by Dr. W. S. Timblin and Dr. C. B. Huggins) (University of Chicago Clinics number 133404), age 16.* For six months the patient had had enlargement of the breasts comparable to that of many adolescent girls. His penis and prostate were normal, axillary and pubic hair abundant, facial hair normal for his age, his voice adult. The testes were small, measuring  $2.5 \times 1.3 \times 1.2$  cm.

In the spring of 1936 a complete 6-day urine collection was secured.

*J. B. (Referred by Dr. W. O. Thompson), negro, age 24.* The patient had mumps orchitis eight years previously and had for seven years recurrent swelling and tenderness of the right testis. Biopsy showed normal testicular tissue. Enlarged painful breasts from which a white discharge could be expressed had been present for one year. He shaved a small circumoral beard twice weekly.

In March 1935 a complete 3-day urine collection was secured.

*T. W. (Referred by Dr. H. T. Ricketts) (University of Chicago Clinics number 116057), age 15.* The patient had breast enlargement for one year, the sub-areolar discs measuring 3 to 4 cm. in diameter. The pubic and axillary hair and the genitalia were normal. There were signs of a mild hyperthyroidism (basal metabolic rate, +18, +19) which subsided under iodine. Nine months later his breasts were smaller. A complete 3-day collection was secured in March 1935.

The data is presented in Table II. We have found no unusual amount of estrogenic material in the urine in any of our four patients (including J. W., the castrate) and hence are unable to bring support to the theory that gynecomastia is due to

hyperestrinism. For two reasons, however, such negative results are not to be considered conclusive. First, samplings of a few days only form a highly imperfect representation of a process that evolves over months and years, and secondly, artificial and hence probably physiological additions of estrogenic material to the organism evoke a notoriously limited urinary excretion. The problem, of course, requires more extensive study. The excretion of androgens was variable.

#### IV. VIRILISM

Virilism has a confusing variety of pathological associations among which no common denominator is as yet apparent. We may distinguish those instances accompanied by (a) ovarian tumors, (b) adrenal tumors, (c) adrenal hyperplasia, (d) pituitary tumors. In certain instances, the adrenal hyperplasia is accompanied by tumors of the anterior lobe of the pituitary body or carcinoma of the thymus. Cushing's theory (20) that basophile tumors of the pituitary body are often responsible for the hypertrophy of the adrenal cortex and the virilism is now well known, much discussed, and still subject to discussion. Crooke (21) has recently found hyaline changes consistently in the basophiles of the anterior lobe in a series of patients with and without adrenal or pituitary tumor, but presenting hypertrichosis, obesity of the face and trunk, hypertension and amenorrhea, thus giving the term "pituitary basophilism" an entirely different significance from that given it by Cushing. So great is the present confusion that the term Cushing's syndrome should be withheld unless an unequivocal pituitary tumor is found since a strictly clinical distinction between his patients and the long known suprarenal virilism has neither been claimed nor shown.

To these four groups must be added a fifth of undetermined pathology comprising by far the majority of those seen. These individuals suffer from hypertrichosis, together on occasion with hypertrophy of the clitoris and often with such substantial disturbances of the menstrual function as oligomenorrhea, amenorrhea and even menorrhagia. Obesity may occur but hypertension and asthenia are rare. The ovaries may be enlarged, sometimes cystic, sometimes fibrotic, the capsules much thickened, changes by no means specific. The adrenals are normal as far as manual ex-



ploration can tell us. Adrenal hyperplasia (22) or occult pituitary or ovarian changes (21, 23) may be present, of course, but the necessary detailed autopsy studies are insufficient to permit full analysis of the problem.

A beginning has been made in the measurement of hormone excretion in virilism. Bühler (24) in 1933 reported 6 to 7 units of comb-growth-stimulating material per day in the urine of a 28 year old girl with hypertrichosis, deep voice, large clitoris, and amenorrhea, by a method which yielded 1 to 2 units per day in normal men. Thirty-five to 40 mouse units of estrogenic material were found. Frank (25) in 1934 reported that in two patients with carcinoma of the suprarenal cortex he had found enormous quantities of estrogenic material in the urine. In the first patient, he records from less than 1,000 to 17,000 mouse units in three-day periods, a total of 57,000 mouse units during a time equivalent to a menstrual cycle. In the second patient, he found 5,000 units per liter. In upwards of 10 more patients without carcinoma, in one of which adrenal cortical hyperplasia was found at operation, he could find no excess. Kurzrok et al. (26) on the other hand, could find but 8 rat units per liter in their case Number 3, a proved carcinoma of the suprarenal cortex, the male-hormone assays on which we are reporting here. Similar values were found in two other girls with hypertrichosis, one with adrenal hyperplasia demonstrable by perirenal air injections. Simpson, de Fremery and Macbeth (27) in 1936 reported their assays on the urine in 12 varied instances of hypertrichosis and included two others examined by Dingemans. By the comb-growth method, five patients showed a marked excess of masculinizing substance and four a more moderate excess. In three cases their values reached from 200 to 500 capon units per day by a technique which gave from 10 to 50 in the normal female. Of especial interest is the finding of between 25 and 50 units per day in the urine of a four-year old pseudo-hermaphrodite. One of their patients with high values was proved to have a carcinoma of the suprarenal cortex. Korenchevsky found in the urine of one of these cases an increase in prostate stimulating power when tested on the rat. The amount of estrogenic ma-

terial did to some extent parallel that of the masculinizing substances. The case of carcinoma of the suprarenal cortex showed, however, less than 600 mouse units per day, and the highest in any case was recorded as "less than 1,440."

Slot (28) has recently described a 49 year old woman with amenorrhea, hypertrichosis and hypertension in which Dingemans found 2,200 international units of comb-growth stimulating material per liter with 100 international units (10  $\gamma$ ) of estrogenic substance. Despite this great excess of male hormone in the urine the adrenal tumor removed contained no more than the livers of normal individuals which served as his control. Saphir and Parker (23) have described a 15 year old girl with hypertrichosis, obesity and amenorrhea who had nests of clear adrenal-like cells in a removed ovary and who excreted 5,000 mouse units of estrogenic substances per liter of urine.

We were able to examine the urine of 16 patients with virilism. They ranged from 13 to 36 years of age; all had striking hypertrichosis, three an enlarged clitoris, and two, hypertension. Excluding the two youngest patients, six had amenorrhea, five irregular menses, and one menorrhagia. Only in two instances was menstruation normal. While several were obese, the rapidly developing facial and trunk obesity, remarked by Cushing, was present in but two (P. R., C. F.) and one of these had purplish abdominal and iliac striae. Two patients had carcinoma of the adrenal cortex proved at operation (G. C., K. P.); two had adrenal tumors proved at autopsy (P. R., C. F.). In seven others, the adrenals were explored surgically and found not demonstrably abnormal. In eight patients without known adrenal lesions, the ovaries at operation were enlarged in six, and in four of these were recorded as cystic. In one patient not operated on, large cystic ovaries were found on pelvic examination. In no case did roentgenograms of the sella turcica show pathology. In five patients, complete urine collections were secured for three or four days; in the remainder for five to ten days. In eight instances, there was no cyclic menstrual bleeding at the time of the urine collection.

*R. Y. (Referred by Dr. C. B. Huggins) (University of Chicago Clinics number 58994), age 18. The patient*

had a boyish figure, hypoplastic breasts, enlarged clitoris and facial, abdominal and limb hypertrichosis. Physical and laboratory examinations were otherwise negative. Scant, transient vaginal bleeding occurred once each at 14 and 17. In 1932, Dr. C. B. Huggins explored the pelvis and found enlarged cystic ovaries which showed on section many primary follicles and numerous cystic spaces.

A complete urine collection for seven days was secured in March 1935 during amenorrhea.

*M. N. (Referred by Dr. A. A. Weinstein), age 21.* The patient had striking facial and abdominal hypertrichosis, normal breasts, blood pressure and pelvic organs. Extensive laboratory examinations were negative. Menses since the age of 14 had recurred every five to eight weeks and lasted five days. Laparotomy in July 1934 at the Vanderbilt University Hospital showed normal adrenals and ovaries.

A complete urine collection for seven days was made in September 1934, beginning ten days after the onset of the preceding menstrual period.

*E. H. (Referred by Dr. W. O. Thompson), age 29.* The patient was somewhat obese with hypertrichosis of the face, chest, abdomen and extremities. Physical, laboratory and pelvic examinations were negative. Since March 1934, when she missed a menstrual period, she has flowed every six weeks. Beginning in September 1934 she flowed continuously for eight weeks. Curettage was then negative.

A complete 3-day urine collection was secured in June 1935. Relation to the cycle was not known to us.

*M. A. (Referred by Dr. W. O. Thompson), age 23.* The patient had hypertrichosis and menorrhagia since the menarche at 19. At operation, both ovaries were one and one-half times the normal size, the capsules much thickened. An operative note states that "exploration revealed no other lesions of note."

A complete 3-day collection of urine during December 1935 was examined. The relation of this collection to the menstrual cycle is not known to us.

*A. D. (Referred by Dr. Edmund Andrews) (University of Chicago Clinics number 149906), age 32.* The patient had great obesity, hypertrichosis of the face, abdomen and extremities, and amenorrhea for 19 months. Her blood pressure was 120/90 and laboratory examination was negative. At operation there was an inflamed periappendicular mass, normal adrenals and enlarged thickened ovaries which showed on section much increase in stroma and little or no follicular activity. Following resection of parts of the ovaries, she bled vaginally.

A complete urine collection for six days was secured before the exploration, during amenorrhea, in April 1936.

*W. K. (Referred by Dr. Henry Jacobs) (University of Chicago Clinics number 135457), age 21.* The patient was an obese girl, with hypertrichosis of the abdomen, extremities and face, hypoplastic breasts, and a deep masculine voice. Since the menarche at 21, the menses

have been very irregular. Physical, pelvic and laboratory examinations have added nothing further.

A complete urine collection for six and a half days was made just before a menstrual period in September 1935.

*E. S. (University of Chicago Clinics number 60628), age 25.* The patient was obese and had hypertrichosis of the face and abdomen, and amenorrhea, with a history of menorrhagia. Physical and laboratory examinations added nothing. Pelvic examination showed enlarged cystic ovaries. In June 1935, Dr. Virgil S. Counsellor operated on her at the Mayo Clinic and resected parts of the large cystic ovaries which on section were recorded as polycystic with oophoritis. The adrenals were not definitely abnormal. An x-ray treatment to the pituitary body was given. In July 1935, she returned to the University of Chicago Clinics (Dr. W. J. Dieckmann) with menorrhagia which subsided after curettage. Brief one-and-a-half-day periods have recurred monthly since.

A 10-day urine collection was secured in January 1935 during amenorrhea.

*G. F. (Referred by Dr. W. O. Thompson), age 17.* She had hypertrichosis of the face, chest, abdomen and extremities. Menses at first every two or three months have been regular recently since taking theelin. Physical and laboratory examinations are negative. At operation (Cook County Hospital) the ovaries were found to be the size of small lemons, "not very cystic" and contained one or more hard nodules which proved to be fibromata on microscopic examination. The ovarian stroma was in part normal and in part fibrotic with evidence of old and recent ovulation present. The adrenals were normal.

We have examined a complete urine collection amounting to five liters collected in March 1934. The time in the cycle is not known to us.

*C. F. (Referred by Dr. Louis Leiter) (University of Chicago Clinics number 141740), age 31.* The patient was obese with swelling especially about the face, with hypertrichosis of the face, abdomen and extremities. Her blood pressure ranged from 150/100 to 220/120. Her previously regular menses had stopped six months before. Pelvic examination was negative. There were no purplish striae, or polycythemia. Osteoporosis and ecchymoses on slight pressure were present. Basal metabolism and sella turcica x-rays were negative. The urea clearance showed good kidney function. Exploratory laparotomy (Dr. D. B. Phemister) in March 1936 revealed normal ovaries and adrenals.

Subsequently the patient's kidney function declined and she died on March 20, 1937, with renal and myocardial failure. Autopsy by Dr. Eleanor Humphreys showed a small pigmented adenoma in the right suprarenal gland with venous thrombosis and focal necrosis; extreme simple atrophy of the left suprarenal gland and of the remainder of the right; focal atrophy and necrosis (x-ray effect?) in the anterior lobe of the hypophysis with focal adenomatous hyperplasia, but without basophile adenoma or basophilic infiltration of the neurohypophysis. There



was widespread atherosclerosis, especially of the small arteries and arterioles; arteriosclerotic atrophy of the kidneys and pancreas; cardiac hypertrophy and dilatation with focal scarring and myomalacia and marked fatty degeneration; visceral chronic passive hyperemia, ascites, hydrothorax and edema of the extremities; extreme fatty infiltration of the liver; atrophy of the thyroid gland with focal adenomatous hyperplasia; atrophy of the uterus and mammary glands; minimal ovarian fibrosis with sparse small follicular cysts and with persistent primordial follicles (without ripening stages); marked generalized osteoporosis with multiple healing rib fractures and collapsed vertebral bodies; hypoplastic bone marrow and lymphoid tissue.

A 6-day urine collection, probably incomplete, was secured in 1936 during amenorrhea, when the urea clearance was normal.

*S. J. (Referred by Drs. S. C. Freed and Samuel Soskin) (University of Chicago Clinics number 156840), age 13.* The patient had hypertrichosis of the face, abdomen and extremities. The breasts were flat, and she had not as yet menstruated. Pelvic examination showed a questionably enlarged clitoris. Blood pressure was normal and physical examination was otherwise negative. Eight months after the urine collection the pelvis was explored by Dr. Karl Meyer at Cook County Hospital who found large cystic ovaries, and no other abnormality.

A complete 7-day urine collection was secured in February 1935.

*R. G. (Referred by Dr. A. K. Koff) (University of Chicago Clinics number 120522), age 32.* The patient had hypertrichosis of the face, abdomen and extremities. She was somewhat heavy and her menses were irregular, occurring as infrequently as eight months apart, but she had given birth to two normal children. Physical examination showed nothing unusual. Pelvic examination showed bilaterally enlarged cystic ovaries.

A complete 7-day urine collection was secured immediately after the close of a menstrual period in October 1935.

*P. R. (Referred by Dr. W. O. Thompson), age 31.* Patient was moderately obese, her plethoric face was covered with a downy growth of hair averaging 3 mm. in length. An excess of hair was also present elsewhere, save for the scalp and axilla, where the amounts were scant. Except for one day's flow ten months previously, she had not menstruated for three years. The labia minora were small, the cervix long and conical, the uterus normal. The skin of the entire body was covered with irregular pink macules with purple striae over the iliac areas and buttocks. Her blood pressure ranged from 182 to 200/126 to 150. Her heart was enlarged, and edema of the feet and spontaneous ecchymoses had been present for six months. The thyroid gland was diffusely enlarged, a tremor of the fingers present; her basal metabolic rate was +18. The urine contained 100 grams of sugar in 24 hours on a diet yielding 200 grams of glucose; her fasting blood-glucose was 170 mgm. per 100

cc., but, curiously, insulin seemed to aggravate her condition. X-rays of the sella turcica showed doubtful erosion. Pyelograms were negative.

She died with thrombotic occlusion of the left external iliac and femoral arteries associated with the heart failure. Necropsy showed cortical adenomata of the right adrenal gland with extensive necrosis and marked atrophy of the cortex of the left adrenal gland; myocardial hypertrophy and degeneration, the thrombotic processes with the resulting gangrene of the left leg, osteoporosis, and "parenchymatous degeneration" of the liver and kidneys. The pituitary was normal macroscopically and microscopically.

We have assayed a complete 3-day urine collection. It was secured during amenorrhea in May 1935.

*G. C. (Referred by Dr. Raphael Kurzrok, Case number 3), age 16.* (Reported in detail by Dr. Kurzrok (26).) Menses began at 13 and after two periods, stopped. Excessive hair appeared on her chest, arms, body, legs and face, the last requiring daily shaving—and her voice deepened. Her clitoris was found to be nearly two inches long. Her blood pressure was 138/76, and an exhaustive laboratory examination was negative save for the beautiful demonstration by x-ray of a mass above the left kidney after the injection of air into the perirenal tissue. Her urine contained no follicle-stimulating hormone and 8 rat units of estrogenic substance. A complete urine collection for eight days was made and the mass then removed. The tumor on section proved to be a carcinoma of the adrenal cortex. Recovery was excellent, and in a month her menses returned, her voice became higher pitched, and her hair began coming off in the bath.

*K. P. (Referred by Dr. Raphael Kurzrok, case number 4, reported in detail by Dr. Kurzrok), age 36.* The patient had suffered from amenorrhea for six years, and from hypertrichosis of the body and face for three years. Her clitoris was very large. X-ray films, after perirenal air injection, showed a mass above the right kidney which was removed at operation. The patient died in post-operative collapse. Autopsy was refused. On section the tumor mass proved to be a carcinoma of the suprarenal cortex. Urine was collected for assay for male hormone for four days before the operation.

*G. N. (Referred by Drs. Russell Wilder and George Crisler).* The patient was obese and had a hypertrichosis of whisker distribution on the face. There were several dark bluish striae on the abdomen and upper thighs. Her menses had been grossly irregular with frequent amenorrhea. She complained of severe headaches and had been in a mental hospital several months of the previous year. Her systolic blood pressure ranged from 140 to 110. Her glucose tolerance gave a diabetic type of curve but otherwise laboratory studies including visual fields and x-rays of the sella turcica were negative. On abdominal exploration, Dr. Waltman Walters found normal adrenals and ovaries and an atrophic uterus.

A 4-day urine collection was secured during amenorrhea in the spring of 1936.

TABLE III  
The excretion of sex hormones in virilism

Patient*	Age	Operation	Adrenals	Ovaries	Menses	Duration of collection	Duration of boiling	Number of capons	International androgen units per day		Theelin per day	Ratio androgens to gamma theelin
									15 minutes	2 hours		
R. Y. <sup>c</sup> ....	18	+		Enlarged cystic	0	7 days	120	7		30	7 4	7.5
M. N.....	21	+	Normal	Normal	Regular	7 days	120	4		16	6	2.7
E. H.....	29	0			Slightly irregular	3 days	120	7		22	12	1.8
M. A.....	23	+	Normal	Enlarged sclerotic	Menorrhagia	3 days	120	7		24	18	1.3
A. D.....	32	+	Normal	Enlarged sclerotic	Amenorrhoea	6 days	15	7	8	5†	10	0.2
W. K.....	21	0			Irregular	6½ days	120	7		44	17	2.6
E. S.....	25	+	Normal	Enlarged cystic	Amenorrhoea	10 days	120	4		23	19	1.2
G. F.....	17	+	Normal	Enlarged cystic	Irregular to regular	5 liters	120	4		34 per liter	11 per liter	3.0
C. F. <sup>h</sup> ....	31	Necropsy	Adenoma	No ripe follicles	Amenorrhoea	6 days (inc.)	15	7	14 per liter	8 per liter †	10 per liter	0.8
S. J.....	13	+	Normal	Enlarged cystic	0	7 days	120	4		16	10	1.6
R. G.....	32	0		Enlarged cystic (pelvic)	Irregular	7 days	120				16	
P. R. <sup>h,s</sup> ..	31	Necropsy	Adenoma	Normal	Amenorrhoea	3 days	120	7		0	4	0.25 -
G. C. <sup>c</sup> ....	16	+	Carcinoma		Amenorrhoea	8 days	120	7		480	8	60.0
K. P. <sup>c</sup> ....	36	+	Carcinoma		Amenorrhoea	4 days	120	7		69		
G. N. <sup>s</sup> ....		+	Normal		Irregular	4 days	15	7	74	44†	14	3.2
M. C.....	25	0			Regular	3 days	60	7	92†	55†	11	5.0
						2 days	60	7	111†	64†	<5	12.8
Average (15 patients (except G. C.)) .....						3 to 10 days	15 to 120	4 to 7		28	11	2.6
Average (4 normals).....						6 weeks	15 to 120	7	47†	28	25	1.1

\* h = hypertension; c = hypertrophied clitoris; s = purple abdominal striae.

† Calculated on the assumption that the 2-hour hydrolysis gives values 60 per cent of those secured by the 15-minute hydrolysis and that the 1-hour hydrolysis gives values 76 per cent of those at 15 minutes. The absolute values for Patient M. C. at the 1-hour hydrolysis are 70 and 84 units. The average ratios given are for the 2-hour hydrolysis.

M. C. (Referred by Dr. Russell Wilder), age 25. Save for the marked hypertrichosis of the face, abdomen and extremities, the patient presented no gross physical abnormality. Her menses, for a while appearing every 15 days, later became of normal periodicity. Pelvic examination was normal and extensive laboratory investigations including an intravenous pyelogram were negative. One of several Ascheim-Zondek tests was faintly positive, and there was on one occasion about 8.5 rat units per liter of estrogenic substances in the urine.

A 3-day and a 2-day urine collection were secured in the spring of 1936.

It will be recalled that in the three normal females studied extensively by seven capon assays, the urine being boiled for two hours, we found from 13 to 46 international androgen units in the 24 hour specimen with an average for each of about 26. Samplings of other normal women and the examination of pooled specimens are in agreement with these values. If we allow for the 40

per cent destruction when compared with the 15 minute hydrolysis, the average becomes 43 and the range 28 to 77. In one normal woman, studied directly by the 15 minute hydrolysis, from 42 to 85 international units were passed, giving an average of 56. Of the fifteen women with virilism studied for male hormone, twelve are within or below this normal range (Table III). Two (K. P., M. C.) are some 25 per cent higher than any normal yet studied. One patient (G. C.), a patient of Dr. Raphael Kurzrok, reported in detail by the Columbia group (26), showed the considerable sum of 480 international androgen units per day (two-hour hydrolysis). She had a proven carcinoma of the adrenal cortex. Furthermore, examination of this urine in the Department of Chemistry by Dr. T. F. Hogness and associates revealed an absorption spectrum unlike that of androsterone and like that of testosterone,

androstene-dione and the adrenal cortical derivative supplied us by Kendall (discussed by the Rochester group (29)). Curiously, we were able to extract no male hormone from the tumor itself.

In the sixteen specimens examined for estrogenic material, no unusually large amount was found, indeed the average normal value was never reached. This may be correlated with the frequent amenorrhea and grossly pathological ovaries often found at operation.

It is difficult to bring regularity and simplicity into this data. A gross excess of masculinizing material is apparently excreted on occasion, in our experience in an instance of adrenal carcinoma. In other patients with virilism there is either no excess or very little. It is thus impossible, at the present time, to support the simple and attractive hypothesis that virilism is due to a hypersecretion of a recognized comb-growth stimulating hormone. The occasional striking positive finding, however, precludes the dismissal of this conception until studies have been made over the long periods during which this syndrome develops. Brief samplings are unfortunately inadequate, especially as the changes of virilism are structural and tend to remain at least for a while after the stimulus evoking them has subsided. Simpson, de Fremery and Macbeth (27) have apparently been more fortunate in their material as they secured a far higher proportion of positive findings. It is well in considering this problem to recall that Frank (25), in studying the urine of patients with adrenal cortical carcinoma, found large amounts of estrogenic material despite the obvious masculinization of the patients. This, too, is irregular, as Slot (Dingemanse) and Kurzrok found normal values. Frank's experience constitutes a curious obverse of the stallion (Zondek (11)) who, with unimpeachable masculinity, excretes much more estrogenic material than the mare.

We have, on occasion, wondered whether the ratio of male to female urinary constituents might not prove a better clue than the gross amounts, as in general normal men excrete more male hormone relative to female than do women. Sometimes this ratio is greatly displaced in the masculine direction (Patients R. Y., G. N. and M. C.), and it is rarely under 1.0 as is frequently true in

the normal woman. This point, however, also awaits further work, especially with reference to the changes accompanying amenorrhea.

The adrenal cortex, of course, is suspected on clinical grounds as a source of the augmented excretion of male sex hormones when this occurs. Recent biochemical work has an interesting bearing on this possibility. Reichstein (30) has separated six crystalline substances from suprarenal cortical tissue. One of these, an unsaturated diketone, of the formula  $C_{18}H_{24}O_3$  ( $\pm C \pm 2H$ ), he reported as one-fifth as active as androsterone in comb-growth stimulating activity. Inasmuch as this observation, as well as the composition and absorption spectrum of the substance, suggest a relation to the ring structure of cholestenone and testosterone, he called the substance adrenosterone. Kendall, Mason and Meyers (29) also obtained a diketone of the formula  $C_{18}H_{24}O_3$  by chromic acid oxidation of their compound E (Wintersteiner's and Pfiffner's compound F). The diketone, when examined spectrographically by Professor T. F. Hogness and his associates in the Department of Chemistry, gave absorption bands like those of cholestenone, testosterone and androstenedione, but unlike androsterone. When tested on the capon, we found it to be one-sixth to one-fourth as potent as androsterone. An oxidation product,  $C_{19}H_{26}O_3$ , prepared by Drs. Oskar Wintersteiner and J. J. Pfiffner from suprarenal cortex and submitted to us for male hormone assay, also gave the same order of activity. The spectrographic findings are especially interesting because they suggest that the androgenic activity and absorption spectrum of the androgenic fraction obtained from the urine of Kurzrok's patient Number 3 are not due to androsterone and dehydroandrosterone, but to an androgenic substance of suprarenal origin which may be closely related to Reichstein's adrenosterone. Its production may be due to a pathological process or perverted metabolism as a result of which such a substance may be formed or be allowed to accumulate in the blood stream to abnormal levels.

**Acknowledgments:** This work could not have been conducted without the cooperation of many interested physicians, each of them aware of the possible significance of assays for these hormones. For the collection

of urine we are greatly indebted to Dr. W. O. Thompson, Cook County Hospital, Chicago; Dr. Raphael Kurzrok, Presbyterian Hospital, New York; Dr. A. A. Weinstein, Vanderbilt University Hospital, Nashville; Dr. Thomas Myers, St. Paul, Minnesota; Drs. Russell M. Wilder and George Crisler of the Mayo Clinic; and the late Dr. James Austin of Columbia, South Carolina. For the placing of their clinical material at our disposal, we are indebted to Dr. S. C. Freed and Dr. Samuel Soskin of Michael Reese Hospital, Dr. I. Becker of the University of Illinois, Dr. M. Kolovros of Gary, Indiana, and Drs. Henry L. Schmitz, W. M. Brunet, W. S. Timblin of Chicago, and Drs. Carl Moore, Percival Bailey, Paul Bucy, C. B. Huggins, Louis Leiter, Edmund Andrews, A. K. Koff, Henry Ricketts and Henry Jacobs of our own staff.

#### SUMMARY

1. Two castrated men excreted traces only of androgenic (comb-growth promoting) and estrogenic substances.

2. Seven eunuchoids excreted on the average a third of the normal amount of androgens, overlapping the normal range on occasion. The output of estrogens was also low. One patient with hypopituitarism excreted small amounts only of both substances.

3. Of four patients with gynecomastia none excreted an excess of estrogenic material. The androgens varied from none at all to a normal amount.

4. Sixteen patients with virilism excreted as a rule normal amounts of androgenic material. A moderate excess of androgens is occasionally found, and the great excess of 480 international units per day was found in one case of carcinoma of the adrenal cortex. The urine of this patient possessed the spectrographic properties of testosterone, androstenedione or cholestenone rather than of androsterone. It was similar in this respect to certain compounds derived from the adrenal cortex. We have had no instances as yet of increased excretion of estrogens in virilism.

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# NUTRITIONAL EDEMA IN THE DOG. V. DEVELOPMENT OF DEFICITS IN ERYTHROCYTES AND HEMOGLOBIN ON A DIET DEFICIENT IN PROTEIN<sup>1</sup>

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In a previous report from this laboratory (1), it was shown that maintenance of dogs on a diet deficient in protein is accompanied by a progressive fall in the concentration of albumin in the serum. Average globulin concentration is virtually unaffected, the decline in albumin being paralleled by a similar decrease in total protein. Studies of nitrogen balance in five animals indicated that the loss of albumin from the serum can account for only 3 or 4 per cent of the total loss of nitrogen from the body. It is reasonable then to inquire what other tissues contribute to the total loss and to what degree. Although a complete answer to this question cannot be given, it has been possible to assemble data which bear on the fate of hemoglobin during maintenance on the diet. Along with the serum albumin, the hemoglobin also suffers depletion; from the quantitative aspect of contribution to the total loss of nitrogen the depletion of hemoglobin is more important than the loss in albumin. The data which lead to this conclusion form the basis of the present report.

## METHODS

*Animals.* Young adult dogs of mongrel breeds weighing between 15 and 25 kilos were selected on the basis of health, vigor and willingness to eat the special diet.

*Diet.* The composition of the low protein diet has been described previously (1); a discussion of the adequacy of the diet from the standpoint of vitamins has also been given (2). From the viewpoint of this paper which is concerned chiefly with hemoglobin it may be noted that, in a control experiment in which 90 grams of the sugar was replaced by an equal amount of casein, there was

no change in relative red cell volume (hematocrit) or in serum protein over a period of 77 days. On the average, the diet as offered furnished 2.4 mgm. of iron per kilo per day; however, after 6 to 8 weeks the intake of iron was usually less because of refusals. As a further control in another experiment the low protein diet was supplemented by the addition of 11 grams of liver extract<sup>2</sup> daily. The changes in this experiment were identical with those in other experiments in which the supplement was not given. It is fair then to assume that the findings to be reported resulted from protein deficiency alone.

*Blood volume.* Plasma volume was estimated by one or another modification of the "dye method" (3). Red cell volume and total blood volume were calculated from the plasma volume by means of hematocrit readings. In most experiments vital red was injected; recently we have used, and now prefer, the blue dye (T-1824, Eastman Kodak Company) described by Gregeresen, Gibson and Stead (4). In our early work dye concentration was measured in the Hilger spectrophotometer as described by Graff and Clarke (5); this method permits separate identification of the color due to dye, of the natural color of the plasma, and of extraneous color arising through hemolysis. The method consumes much time and in our hands has not brought increased accuracy over that obtainable in a colorimeter when certain precautions are observed. The first precaution is that the technic of blood sampling be such that hemolysis of red cells is

<sup>1</sup> The results of this investigation were presented in less complete form at the meeting of the American Pediatric Society, Bolton Landing, New York, June 13, 1936 (Am. J. Dis. Child., 1936, 52, 1280).

<sup>2</sup> The liver extract was Liver Extract Number 343 furnished through the kindness of Eli Lilly and Company. Cod liver oil was given in all diets in the form of Cod Liver Oil Stearine contributed by Mead Johnson and Company. In a few experiments the diet was supplemented by the addition of a concentrate of rice polishings known by the trade name of Ryzamin-B and donated by Burroughs Wellcome and Company.

avoided or reduced to a very slight amount. Frequent examination of plasma in a Bausch and Lomb spectrometer has demonstrated the feasibility of obtaining a majority of samples without contamination by hemoglobin. When slight hemolysis has occurred and the blue dye, T-1824, is being used it is possible to eliminate that portion of the spectrum in which the absorption of light by hemoglobin is strongest by using a red filter, Wratten No. 72, in the colorimeter. The second precaution is that the known and unknown mixtures of dye and plasma which are to be compared in the colorimeter should match as closely as possible. To secure close matching it is frequently necessary to prepare a second standard after the result of comparing the first with the unknown mixture has been obtained. In general, the necessity for close agreement between the two mixtures increases in proportion to the intensity of natural plasma color. The colorimeter comparison is made with the purpose of ascertaining the factor which relates concentration of dye in the unknown mixture *a* to concentration of dye in the known mixture *b*—that is, one seeks for the ratio of *a* to *b*. Actually since both mixtures contain an equal amount of plasma, the natural color *c* of which is variable, the colorimeter gives the ratio of  $a + c$  to  $b + c$ . It is clear that this latter ratio approaches the value of the desired ratio under two conditions, namely, when natural color *c* is very small and when the two solutions nearly match so that both ratios approach unity. Dye injections were made into the jugular vein; samples for analysis were withdrawn from the femoral artery and mixed under oil with a measured amount of 1.4 per cent solution of sodium oxalate. The amount of blood in the mixture was determined by weighing. This part of the procedure was the same as that described by Graff and Clarke (5); the technic of these authors was also followed in making the hematocrit estimations.

The determination of plasma volume and blood volume is admittedly not a precise analytic procedure; the exact error is not known. In order to minimize the influence of the error the average of findings in a number of experiments will be reported rather than the measurements on single animals. The significance of such averages can be established by statistical methods even though the error of single measurements is unknown.

#### REPRESENTATIVE PROTOCOL

The data upon which this report is based represent serial observations in 81 experiments performed on 38 dogs. The duration of the experiments was from 21 to 108 days. Approximately 140 measurements of blood volume were utilized in calculating the average values which are to be given. Since so much observational data cannot be presented in detailed form, the following single protocol is offered as representative of the longer type of experiment from which the data were assembled.

Dog 5-69, a male of police type, aged about 1 year, was placed on the basal low protein diet supplemented by the addition of 90 grams of casein daily on March 4, 1935. The diet was well taken and resulted in a rapid gain of 3 kilos; the feeding of casein was continued until the weight had been stationary for 8 days. On April 1 (first experimental day) the casein was removed from the diet and the period of low protein feeding commenced at a level of 75 calories per kilo of weight per day. The basal diet was supplemented by the daily addition of 0.4 gram of a concentrate of rice polishings and of 11.0 grams of liver extract. There were no refusals until the 53d experimental day. At this time the appetite began to fail; on the 65th day the diet was reduced to one-half the original quantity although the supplements were continued in the original amounts. This level of feeding was maintained throughout the remainder of the experiment; because of anorexia the diet often had to be fed forcibly. Edema first appeared over the dorsa of the hind feet on the 59th day. During the next 10 days the edema increased in amount and extended to involve all four legs. Thereafter it fluctuated in amount but gradually became massive in the hind legs. Ascites did not develop and the edema of the forelegs was always moderate. The animal was sacrificed on the 101st day by injecting ether into the heart. During the experiment the following measurements were made:

Albumin per 100 cc. serum: 1st day, 3.39 grams; 22d day, 2.65 grams; 43d day, 2.20 grams; 60th day, 1.66 grams; 64th day, 1.58 grams; 72d day, 1.54 grams; 87th day, 1.24 grams.

Relative red cell volume: 1st day, 42.3 per cent; 22d day, 45.1 per cent; 43d day, 41.8 per cent; 47th day, 41.0 per cent; 68th day, 34.7 per cent; 72d day, 32.6 per cent; 89th day, 32.2 per cent.

Erythrocytes per cubic millimeter of blood: 1st day, 5.38 million; 29th day, 6.33 million; 50th day, 5.56 million; 68th day, 4.62 million; 80th day, 5.10 million; 87th day, 5.13 million.

Hemoglobin per 100 cc. blood: 1st day, 14.5 grams; 29th day, 17.0 grams; 50th day, 14.5 grams; 68th day, 11.6 grams; 80th day, 12.2 grams; 87th day, 10.9 grams.

Plasma volume: 1st day, 744 cc.; 22d day, 636 cc.; 43d day, 676 cc.; 72d day, 687 cc.



Erythrocyte volume: 1st day, 546 cc.; 22d day, 522 cc.; 43d day, 486 cc.; 72d day, 331 cc.

Body weight (actually determined daily): 1st day, 16.2 kilos; 20th day, 15.0 kilos; 40th day, 14.5 kilos; 60th day, 14.0 kilos; 80th day, 12.6 kilos; 100th day, 11.6 kilos.

#### EXPERIMENTAL OBSERVATIONS

*Effect of the low protein diet on relative red cell volume and hemoglobin.* Direct measurement of hemoglobin has not been a routine procedure in the conduct of these experiments; however, measurements of relative red cell volume in hematocrit tubes have been made regularly. There are 21 protocols with sufficient data to permit following the course of this measurement through a period of 80 to 90 days on the diet. The findings are presented in Figure 1 in the form of an average

the final value at the end of 3 months is as high as it is, namely, 72 per cent of the initial level.

Since there is no obligatory parallelism between relative red cell volume and hemoglobin, four animals were selected for a more intensive study in which erythrocyte counts, hemoglobin determinations, and hematocrit readings were made at regular intervals during maintenance on the diet. From the data obtained the value for each of the three measurements at 10-day intervals was determined by interpolation; the resulting values for each of the 4 dogs were averaged; these average figures were finally expressed as percentages of the mean level during the entire experiment. This method of expressing the results permits one to chart the three measurements on the same scale

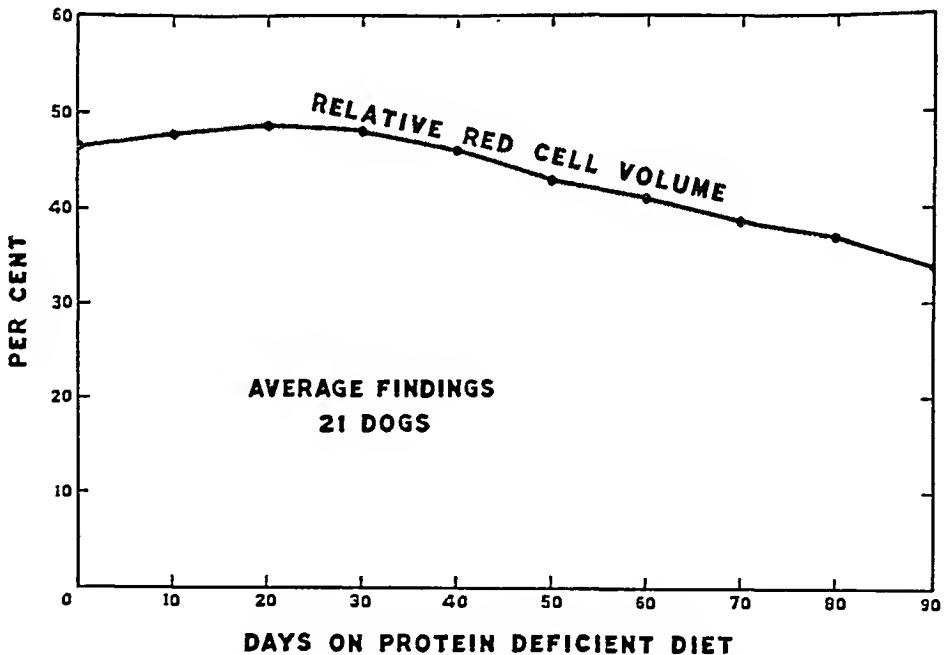


FIG. 1. THE AVERAGE RESULT OF HEMATOCRIT READINGS IN 21 DOGS DURING MAINTENANCE ON THE LOW PROTEIN DIET

curve for the 21 animals. The average relative red cell volume increases during the first 20 to 30 days of maintenance on the diet and thereafter declines gradually but progressively. The initial value is 46.6 per cent cells; the last value recorded after 90 days on the diet is 33.7 per cent cells. The conclusion is warranted that maintenance on the diet eventually leads to a decrease in relative red cell volume but it is interesting that

so that changes in one measurement which are exactly proportional to changes in another will produce identical lines on the chart. Figure 2 presents the findings with these animals. The three curves representing hematocrit, hemoglobin and number of erythrocytes, although not identical, do follow the same trend with sufficient closeness to make it clear that variations in relative red cell volume in these experiments provide a satis-

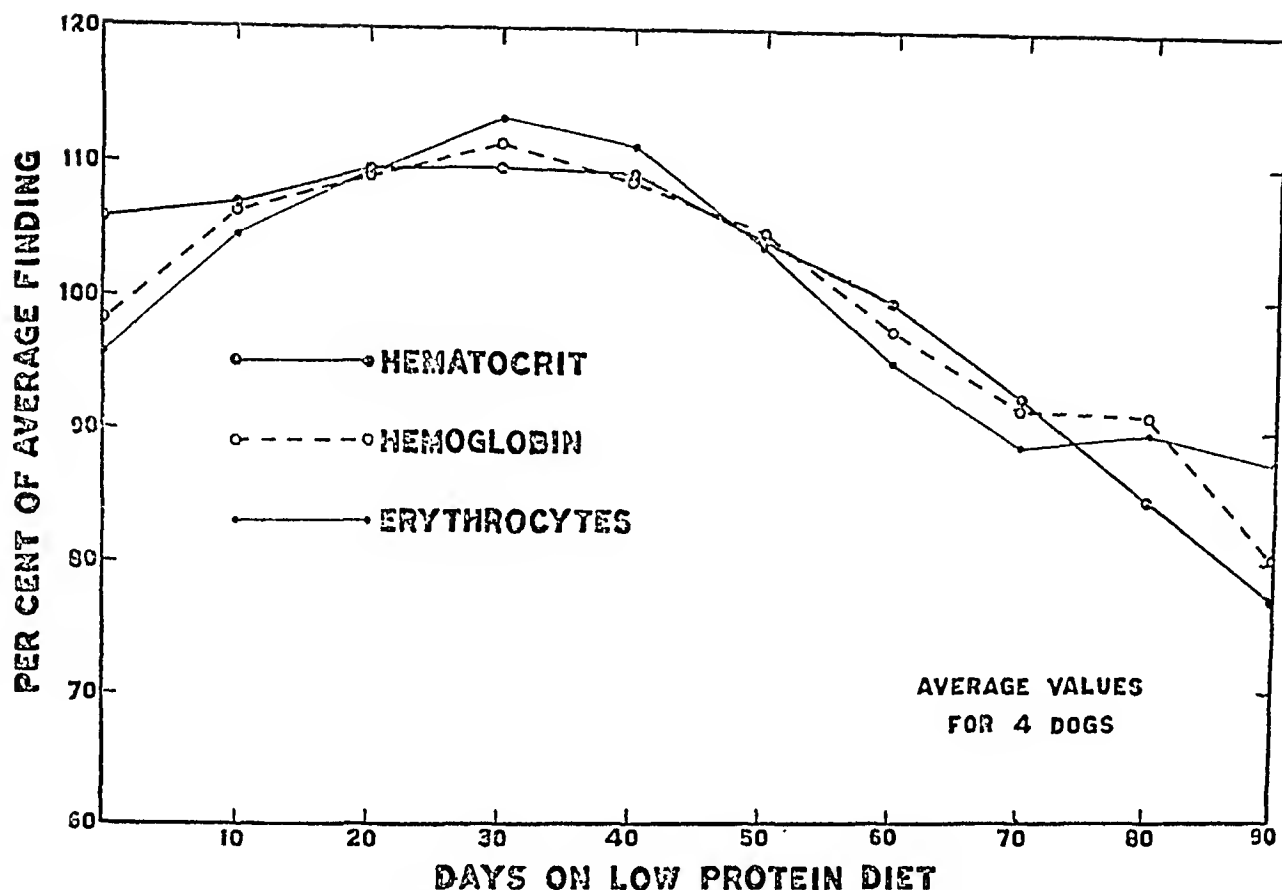


FIG. 2. RELATIVE VARIATIONS IN PER CENT CELL VOLUME, HEMOGLOBIN CONCENTRATION AND NUMBER OF ERYTHROCYTES IN 4 DOGS DURING MAINTENANCE ON THE LOW PROTEIN DIET

factory measure of associated variations in hemoglobin and red cell count. This conclusion is supported by the failure to observe consistent changes in the color index during the experiments. The three curves in Figure 2 follow the same general course as the line in Figure 1, that is, there is an initial rise which reaches a peak in about 30 days and thereafter a progressive fall.

*Effect of the low protein diet on the volume of plasma and red cells in the circulation.* An explanation of the initial rise in hemoglobin as well as a better measure of the final depletion are forthcoming when one examines data obtained in measurements of blood volume. The average findings with ten dogs are presented in Figure 3. The total volume of circulating erythrocytes declines progressively during maintenance on the diet; there is no initial rise and the final volume which is reached after 80 days is about half (53 per cent) of the initial. Extended to 90 days by extrapolation, for comparison with Figure 1, the data permit an estimate of about 46 per cent of the total red cells remaining in the circulation for this length of time; the estimate from Figure 1

which was based solely on concentration of red cells was 72 per cent and was far from the true degree of hemoglobin depletion. Figure 3 also records a progressive decline in total blood volume during maintenance on the diet. However, the plasma volume decreases during the initial 20 to 30 days only; thereafter, it is maintained at an approximately constant value. Because the early decline in plasma volume takes place at a more rapid rate than the decrease in cell volume it follows that the concentration of red cells must rise during this period. The data therefore offer an explanation for the early increase in concentration of red cells and hemoglobin which was shown in Figures 1 and 2.

That deficits in serum albumin are associated with diminished volume of the plasma has been established by Darrow and Buckman (6) for children with nephrosis, by Chang (7) for humans with nutritional edema, by Lepore (8) for hypoproteinemic dogs, and by others. The results in these experiments are therefore in agreement with data recorded elsewhere. The fact that the plasma volume does not continue to fall

after 20 to 30 days on the diet does not mean that at this stage it is no longer sensitive to changes in albumin concentration. At any stage of serum albumin depletion an immediate rise in plasma volume will occur if the albumin concentration is raised by transfusions with serum (9). The

by the serum protein concentration only insofar as the cell volume is constant."

*Depletion of hemoglobin during the early stages of maintenance on the low protein diet.* From Figure 3 it is seen that the diminution in volume of red cells and therefore in hemoglobin appears

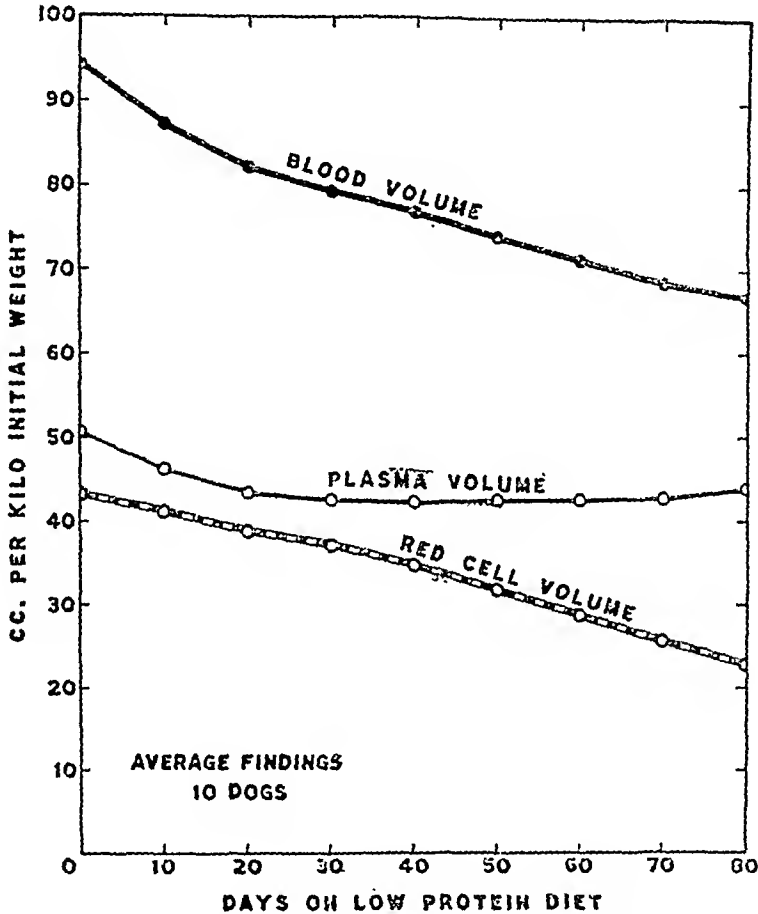


FIG. 3. CHANGES IN BLOOD VOLUME, PLASMA VOLUME AND RED CELL VOLUME DURING MAINTENANCE ON THE LOW PROTEIN DIET

maintenance of plasma volume at a constant level during the later stages can only mean that another limiting factor has come into play, namely, the total blood volume. Since the loss of red cells appears to go on relentlessly it is perhaps not surprising that further reductions in blood volume should be opposed by a force strong enough to compensate for the effect on plasma volume of increasing albumin depletion. Melnick and Cowgill (10) have observed an identical phenomenon in dogs subjected to plasmapheresis; they state that "the plasma volume appears to be regulated

to begin at once when the dietary protein is insufficient. Because this circumstance implies the absence of a reserve store of hemoglobin in the body, it is worth while to establish the existence of an early change by rigid statistical methods. Observations in 60 experiments are available for this purpose. With these animals measurements were made initially and again after 3 weeks on the diet. The average findings shown in Table I are in agreement with the corresponding portion of the curves in Figures 1, 2 and 3. During the 3 weeks there is a rise in relative red cell volume and a fall

TABLE I

Average change in the blood of 60 dogs after maintenance for 3 weeks on the low protein diet. Average weight of the dogs = 19.3 kilos

	Hematocrit	Total circulating volume		
		Plasma	Red cells	Blood
	per cent	cc.	cc.	cc.
Initial . . . . .	47.5	935	859	1793
3 weeks . . . . .	49.4	817	811	1628
Change . . . . .	1.9	118	48	165

in plasma volume, in red cell volume and in total blood volume. The average decrease in red cells is 47.6 cc. or 5.5 per cent of the average initial cell volume. The decrease of 47.6 cc. possesses a probable error of  $\pm 6.25$  cc.; the ratio of the decrease to its probable error is 7.6 and indicates that the probability of a decrease being due to errors of sampling and measurement is less than one in a million.

Depletion of hemoglobin then is clearly demonstrable in as short a time as 3 weeks when the dietary protein is inadequate. Indeed, there is no evidence in the experiments that the depletion does not begin at once, that is, there is no evidence of a reserve supply either of hemoglobin or of precursors of hemoglobin which are being utilized to maintain a constant amount in the circulation. If such reserves are called upon their effect must be offset by other factors within a very few days. This finding has at least theoretical interest since Whipple and his collaborators (11) have demonstrated clearly that the body does contain a reserve supply of hemoglobin-building material which can be mobilized to assist in recovery from the anemia which follows hemorrhage. In the present experiments the failure of normal hemoglobin production is probably not concerned with the chromogenic iron-containing portion of the hemoglobin molecule; it probably results from insufficiency of the protein, globin. Presumably all of the body proteins, and among them globin, are affected by the faulty diet. Moreover, it would seem that the reserve stores of protein, which under acute stress are capable of yielding new hemoglobin, are gradually depleted along with the globin and other body proteins. Thus one observes no evidence of reserve hemoglobin material in experiments of this type. The point is worthy of

emphasis because the experimental conditions are such as must often be duplicated in the clinic.

*Relative aspects of the depletion of the circulating proteins.* By combining the data from a previous article (1) concerning the fall in concentration of albumin and globulin in the serum during maintenance on the diet with the findings in this investigation of the changes in the circulating volumes of plasma and of red cells, it is possible to determine the relative extent to which the several circulating proteins are depleted. The result of such a calculation is presented in Figure 4. The albumin of the serum suffers the greatest percentage depletion; after 80 days only 30 per cent of the original amount remains in the circulation. With hemoglobin, about 50 per cent remains. The losses contrast sharply with the slight degree to which the serum globulin is affected; 90 per cent of the initial supply is still circulating at the end of the same period. Figure 4 also shows the average percentage loss in body weight during the experiments. Although the meaning of this weight line is somewhat confused by the tendency toward edema formation, it is probably sufficient to show that the losses in hemoglobin and in serum albumin are greater, and the loss in serum globulin is less, than the loss in total body mass.

The percentage losses shown in Figure 4 do not indicate the actual quantities of the several proteins which are removed from the circulation. The distinction assumes importance from the circumstance that a healthy dog has in his circulation about nine times as much hemoglobin as serum albumin. The 50 per cent loss of hemoglobin therefore refers to a much larger amount of protein than the 70 per cent loss of albumin. From the data obtained in the experiments in which simultaneous measurements of relative red cell volume and hemoglobin were carried out, it is estimated that the average initial level of hemoglobin in the ten dogs of the blood volume series was 15.3 grams per 100 cc. of blood. The corresponding average for blood volume was 93 cc. per kilo and for body weight it was 16.7 kilos. From these data the average total circulating hemoglobin per dog can be computed; it was 237 grams. Since 47.2 per cent (from values in Figure 4) of this hemoglobin disappeared during a period of 80 days on the diet, the total loss in

hemoglobin per dog is found to be 112 grams. In a similar way the loss in serum albumin is found to be 18.3 grams and the loss in serum globulin is 2.4 grams.

is shown graphically in Figure 5. Roughly one-fifth of the body protein which is catabolized by the dog during this period of insufficient protein intake is withdrawn from the circulation; the re-

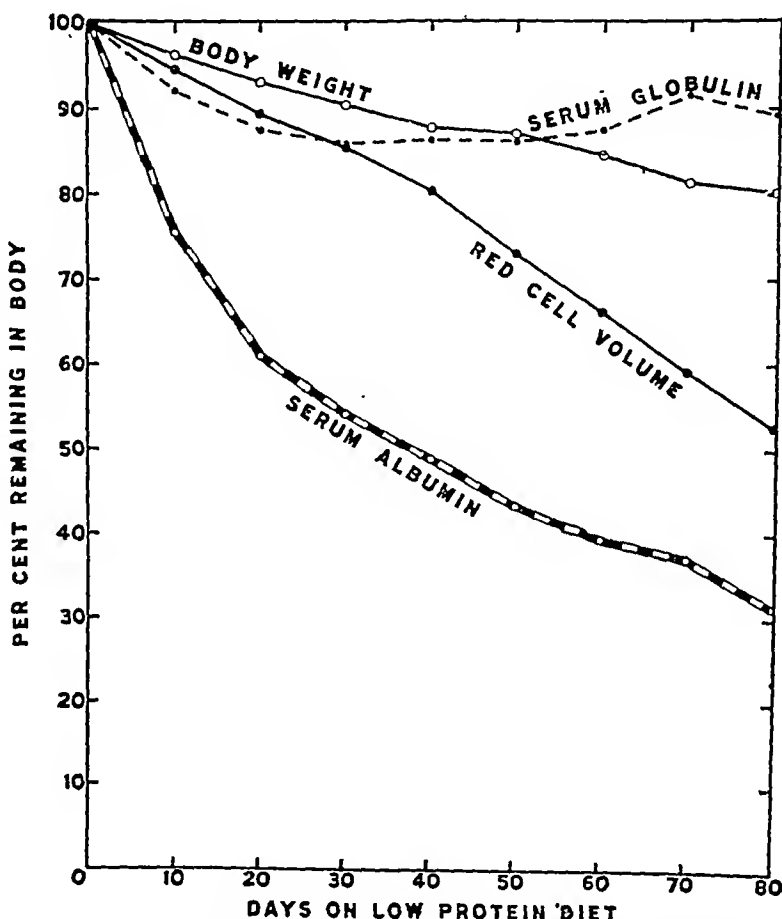


FIG. 4. LOSSES IN THE CIRCULATING PROTEINS DURING MAINTENANCE ON THE DIET EXPRESSED AS PERCENTAGES OF THE ORIGINAL AMOUNT REMAINING AFTER VARIOUS TIME INTERVALS

The results of the preceding calculation are conveniently expressed in relation to an approximation of the total nitrogen loss from the body during the same period. From the results of studies of the nitrogen balance in another series of five dogs on the same diet (1), we have estimated an average daily loss of nitrogen of 1.2 grams for dogs whose weights correspond to those in the present series. This means a loss of protein of 7.5 grams per day or a total loss during 80 days of 600 grams of protein. The relationship between this approximation of the total loss of protein and the losses in circulating proteins

maintaining four-fifths is contributed by tissues outside the circulation. Within the blood stream the relative contributions are: hemoglobin, 18.7 per cent; albumin, 3.1 per cent; globulin, 0.4 per cent. Moreover, the circumstance that the several circulating proteins are involved both relatively and absolutely to different extents in the process of protein catabolism suggests strongly that the proteins of organs and other body tissues are likewise involved to different degrees. This is in agreement with the recent experiments of Addis, Poo and Lew (12) who have shown with rats that during a seven-day fast the liver loses 40 per

cent of its original protein, the kidneys and the blood 20 per cent each, the heart 18 per cent, the brain 5 per cent, and that there is no loss of protein from the eyes, the testicles or the adrenals.

that the formation of erythrocytes and the production of hemoglobin are essentially independent physiological processes, then it must be admitted that these dogs do not build red cells in a normal

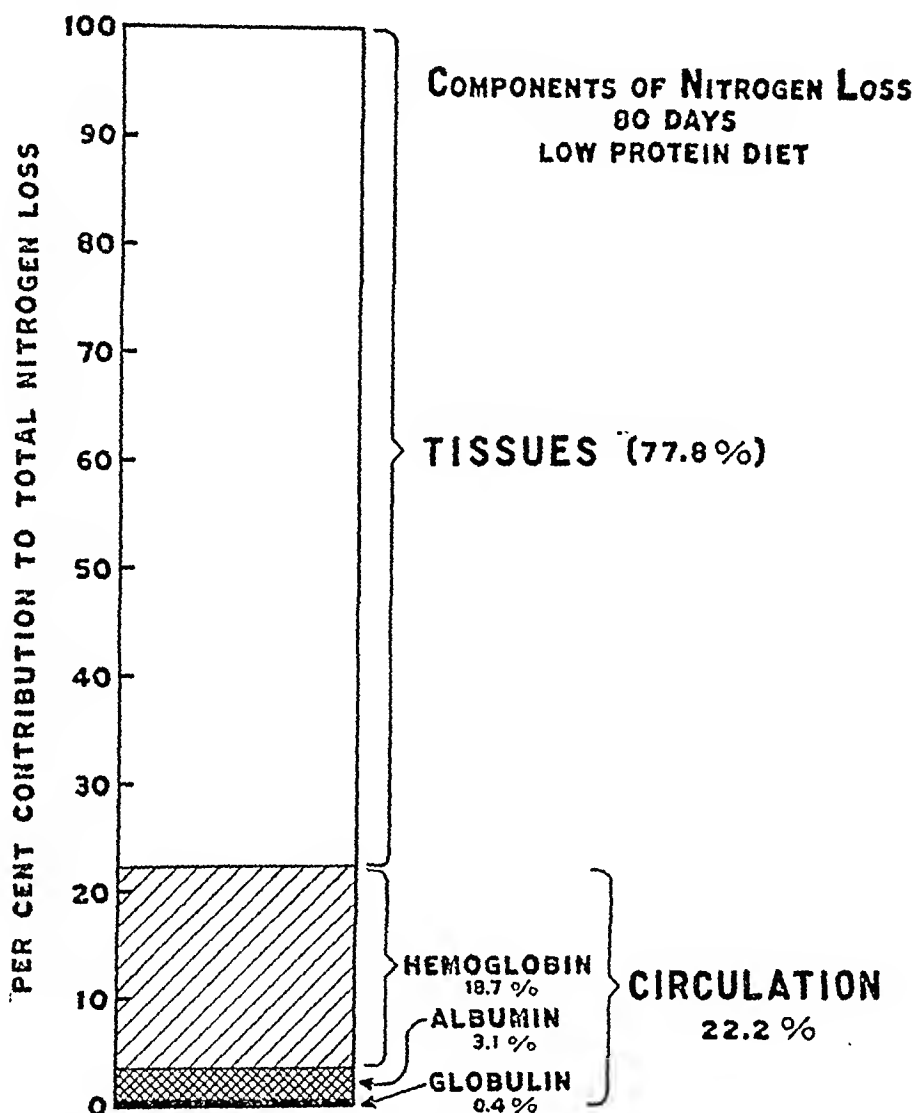


FIG. 5. RELATIONSHIP BETWEEN THE TOTAL LOSS OF PROTEIN FROM THE BODY AND THE LOSSES IN THE CIRCULATING PROTEINS AFTER 80 DAYS ON THE LOW PROTEIN DIET

#### COMMENT

When the investigations reported in this paper were discussed at the meeting of the American Pediatric Society, Cooley (13) objected to interpreting the experiments as indicating defective formation of hemoglobin. Since, during maintenance on the low protein diet, the changes in the hemoglobin level parallel those of the erythrocyte count, Cooley thought there was an equal probability that the defect was one which concerned the structural material for cell building. This possibility cannot be denied. Indeed, if it is true

way. If they did, one would expect to find a secondary anemia of the type which results from deprivation of iron, that is, an anemia with low hemoglobin, relatively high erythrocyte count, and low color index. However, the low protein diet is known to produce general depletion of the stores of protein in the body; for this reason it is difficult to believe that the depletion of hemoglobin is entirely secondary to an inability of the body to manufacture the structural material of cells. It is easier to think that the formation of both erythrocytes and hemoglobin is inhibited by

the diet. The point which Cooley has raised is of considerable interest. It is not, however, concerned with the basic fact that dogs during maintenance on a low protein diet lose a large amount of hemoglobin and that this loss constitutes an appreciable portion of the total nitrogen which is removed from the body.

From the clinical standpoint this work has served to stress again the important relationship which exists between the total volume of the plasma and its albumin concentration. It further emphasizes the fact that the usual hemoglobin determination is liable to give a false picture of the total hemoglobin when the conditions are such that the plasma volume is changing. Many patients with illnesses of brief duration experience anorexia and as a result show a moderate depletion of albumin in the serum. With these patients the hemoglobin level is usually unaffected; with more protracted illness anemia is a common sequel. In the light of this work it appears likely that this early maintenance of a normal concentration of hemoglobin in the blood should be interpreted not as an evidence of a "reserve store" of hemoglobin in the body but as an expected phenomenon when both erythrocytes and the volume of the plasma are decreasing at the same rate. In another group of patients it is sometimes necessary to interpret correctly the significance of a rapid fall in hemoglobin concentration at a time when the volume of the plasma is increasing rapidly. This phenomenon is encountered in the nutritional type of hypoalbuminemia; with the institution of high protein feeding the serum albumin and with it the plasma volume increase rapidly, and the hemoglobin declines. In one patient whom we were permitted to observe, the fall in hemoglobin was so marked, from 14.4 to 9.8 grams per 100 cc. blood after four weeks of high protein feeding, that her attending physician became alarmed and ordered a transfusion. With this patient the decline in the hemoglobin level was associated with a rise in the concentration of protein in the serum from 3.2 to 5.4 grams per cent; it is almost certain that the hemoglobinemia resulted from the increase in plasma volume which accompanies a rising albumin concentration and that it was not an evidence of disappearance of hemoglobin from the body.

#### SUMMARY

Dogs maintained on a diet deficient in protein exhibit in the blood an initial rise and later a progressive fall in relative red cell volume, in red cell count, and in hemoglobin concentration. Measurement of the total volume of red cells in the circulation shows that the decline in hemoglobin is continuous from the time of starting the diet. Measurement of the total amount of plasma in the circulation discloses an initial rapid fall to a volume which is then maintained for the duration of the experiment. Because the early decreases in circulating volumes affect both plasma and erythrocytes, the usual red cell counts and hemoglobin determinations fail to record the progressive loss from the circulation.

The decrease in volume of the red cells is demonstrable with statistical certainty after maintenance on the diet for as short a time as 3 weeks. This circumstance argues against the existence of a "reserve store" of hemoglobin-building material which can be utilized to maintain a constant level of hemoglobin in the blood. Since such a reserve is clearly demonstrable when the hemoglobin is depleted by hemorrhage, it appears that not only the circulating hemoglobin but also the reserve stores are depleted by the diet.

When the loss of the circulating proteins during a period of 80 days on the diet is expressed as a percentage of the original quantity present, it appears that serum albumin suffers the greatest depletion, that hemoglobin is somewhat less involved, and that serum globulin is very slightly affected. When the loss is considered in terms of absolute quantity of protein removed from the circulation, it is six times as great for hemoglobin as for serum albumin and seven times as great for serum albumin as for serum globulin. When the loss of protein from the circulation is compared with the total loss of protein from the body, it appears that about one-fifth of the total loss is from the circulation and that about four-fifths is from tissues outside the blood stream.

It is pointed out that the depletion of hemoglobin brought about by the diet may not result entirely from a defect in the ability to form hemoglobin but that there may also be a failure to produce the structural material for building red cells. A group of clinical patients is cited in whom the

results of serial hemoglobin determinations can be interpreted in the light of findings in this investigation. These are patients who are developing or recovering from deficits in serum albumin and in whom the hemoglobin level will be affected by the associated changes in plasma volume.

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# LOSS OF MINERALS THROUGH THE SKIN OF NORMAL HUMANS WHEN SWEATING IS AVOIDED

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Water is constantly vaporizing from the human skin surface as an important part of the heat regulatory mechanism. There are two sources for water to be vaporized: there is always vaporization which occurs as a result of the water-rich human mass existing in a relatively dry atmosphere. Water lost in this way is spoken of as "insensible loss of water." When the amount of heat carried away by this process is insufficient to maintain the homeothermic state, an additional supply of water is pumped onto the surface of the skin by the sweat glands to aid in the cooling process.

There have been many analyses of sweat; and quantitative studies of water and minerals lost through the skin *during sweating* have also been made (1, 2, 3, 4, 5, 6). Usually in studies of the latter type, one does not know how much of the total loss of water and inorganic salts is from the sweat glands. There is, in the literature, insufficient data regarding the amount of minerals lost through the skin when sweating is avoided. Mention of the most noteworthy of such data follows. McCance (7) in his studies of sodium chloride deficiency in man has taken into account the loss of minerals and nitrogen in the "insensible perspiration" both during salt restriction and recovery. Cole and Curtis (8) have measured the iodine lost through the skin in some of their iodine balance studies. Swanson and Iob (9) measured the cutaneous loss of some inorganic elements in babies 2 to 24 weeks old, and although the subjects were kept in rooms with the temperature maintained almost constant between 75 and 78° F., the elimination of elements "in sweat" is reported. These workers determined the daily loss of sodium, potassium, calcium, phosphorus and chlorides and found that the amount of potassium lost through the skin was more than 30 per cent of the "retention" calculated in the usual manner. Neglecting loss through the skin caused consider-

able error in the sodium and chloride balances also, but the calcium and phosphorus losses through the skin were negligible.

With the exception of the work of McCance (7) none of the literature cited gives us any information regarding the cutaneous loss of minerals from the healthy adult man who is not sweating. We have therefore conducted the following investigation in order to obtain such information.

## EXPERIMENTAL

The subjects for the experiments were two healthy adult men who went about their usual laboratory duties with special precautions only to keep themselves cool enough to prevent sweating. Special clothing worn during the experiment consisted of medium weight cotton underwear and socks which had previously been thoroughly washed in distilled water until free of chloride. This clothing covered the entire body with the exceptions of the hands, head and neck. No special precautions were taken with the outer clothing except that new shoes were worn. Food and water were uncontrolled except that during the second experiment on Subject B and for the three preceding days the intake of sodium chloride was rigidly restricted.

After a cleansing bath with soap and tap water the subject bathed in three successive changes of distilled water rubbing himself vigorously with a wash cloth during each washing, then dried himself. The wash cloth and towels used had previously been treated to remove all soluble minerals. He then donned the underwear, the socks, and his regular clothing and went about his daily routine. Twenty-four hours later the three baths in distilled water were repeated in the same way and all washings saved. The underwear, socks, wash cloth and towels used during the experiment were washed again in distilled water and all washings combined with the bath water. The combined washings and bath water were evaporated to less than a liter and the insoluble material removed by centrifuging. The precipitate was washed four times and the washings added to the soluble fraction. This solution was then evaporated to a small volume and finally made to 100 cc. Aliquots were used for the following determinations: sodium, Butler and Tut- hill (10); potassium, Shohl and Bennett (11); chloride by a modified Vohlhard-Harvey titration (12) after removal of the silver chloride by filtering; sulfate was

TABLE I

*The cutaneous elimination of sodium, potassium, chloride, sulfate sulfur and nitrogen, and the urinary excretion of sodium and chloride during the 24-hour studies*

	Subject A		Subject B	
	1	2	1	2
	mgm.	mgm.	mgm.	mgm.
Cutaneous excretion				
Sodium.....	209	182	71	131
Potassium.....	148	133	140	179
Chloride.....	248	220	146	153
Sulfate sulfur.....	60	106	95	91
Nitrogen				
Soluble.....	180	190	200	230
With the precipitate...	74	330	100	184
	grams	grams	grams	grams
Urine				
Sodium.....			3.749	0.798
Chloride.....	11.289	8.662	6.780	1.818

weighed as barium sulfate; nitrogen by modified Pregl micro-Kjeldahl (13); calcium, by the method of Shohl and Pedley (14); and phosphate by the Fiske-Subbarow method (15).

The same methods were used for the estimation of sodium and chloride of the urine collected during the experiment.

Two 24-hour experiments were carried out on each of the two subjects. The results of these experiments are recorded in Table I. The sodium of the bath water varied from 71 to 209 mgm.; the potassium was 133 to 179 mgm.; the chloride was 146 to 248 mgm.; and the sulfate was 60 to 106 mgm. of sulfur. Neither calcium nor phosphate was found. No attempt was made to balance the anions and cations by the determination of other metals or acids or of pH. In the precipitate, which is made up of lint from the towels and clothing along with variable amounts of cellular debris and hair, the amount of nitrogen would be expected to vary considerably. The nitrogen in solution was found to be 180 to 230 mgm.

#### DISCUSSION

It had previously been found by Wiley, Wiley and Waller (16) that Subject B over a period of 77 days excreted an average of 37 mgm. of sodium per day. The same subject lost 71 and 131 mgm. of sodium daily through the skin during the two experiments reported here. These values illustrate the relative importance of measuring the sodium lost by the two paths of excretion. During the low intake of sodium chloride, particularly, (second experiment on Subject B), cutaneous excretion cannot be disregarded, for in this experiment more than 14 per cent of the total urinary and cutaneous sodium is lost through the skin.

This experiment also shows that the loss of sodium, chloride and other minerals through the skin does not vary with the intake of sodium chloride or with the amount in the urine.

It is interesting to compare our findings with those reported by McCance (7). During a "recovery period" of 5 days one of McCance's subjects lost 370 mgm. of sodium and 670 mgm. of chloride in the "insensible perspiration." These values are equivalent to 74 mgm. of sodium and 134 mgm. of chloride per day. Another subject lost 98 mgm. of potassium per day in her "insensible perspiration." The amounts are of the same order but lower than those reported here.

Even though special care was taken to avoid sweating, we wished to prove that the inorganic elements found in these studies did not come from unnoticed sweat. Further, we wished to eliminate the possibility that inorganic salts might have reached the garments worn from a source other than the skin. Accordingly a 12-hour experiment was carried out while Subject B lay nude on a rubber sheet in a secluded room in which the temperature was maintained at 25 to 28° C. During this period there was no detectable moisture on the skin. The insensible loss of weight for this 12-hour period, determined by the method of Newburgh, Wiley and Lashmet (17), was 397 grams. Sixty-two grams were lost due to the difference in weight between outgoing CO<sub>2</sub> and incoming O<sub>2</sub>. The remainder, 335 grams, was water vaporized from the lungs and skin. Under the prevailing conditions about  $\frac{2}{3}$  of the water vapor comes from the skin, thus about 225 grams of water was vaporized from the skin. It carried with it 32 mgm. of sodium, 47 mgm. of potassium, 53 mgm. of chloride and 34 mgm. of sulfate sulfur. Comparing these 12-hour values obtained during rest with those for Subject B in Table I, and realizing that the insensible loss of water is considerably greater when the subject is up and about, it is obvious that little if any of the 24-hourly values of the substances studied could have come from sweat or sources other than the skin.

According to Fishberg and Bierman (5) sweat contains about 1880 mgm. of sodium, 3000 mgm. of chloride and 200 mgm. of potassium per liter. Then insensible water contains a much lower con-

centration of sodium and chloride and approximately the same concentration of potassium as is found in sweat. The higher K:Na ratio of insensible perspiration as compared with sweat has been pointed out previously (4, 6).

#### SUMMARY

Experiments were conducted to measure the loss of certain inorganic elements through the skin of two healthy adult men while precautions were taken to prevent sweating. No calcium or phosphorus was found. The 24-hourly eliminations of the other substances studied were: sodium 71 to 209 mgm., potassium 133 to 179 mgm., chloride 146 to 248 mgm. and sulfate sulfur 60 to 106 mgm. The cutaneous loss of sodium and chloride did not vary with the intake or urinary output of sodium chloride.

The amounts involved are of considerable importance for the accurate determination of the exchange of these elements as commonly done in mineral "balance" studies, and unless the cutaneous excretions are actually measured, suitable additions should be made to the outgo to correct for losses through the skin.

The authors gratefully acknowledge the many helpful suggestions made by Dr. L. H. Newburgh throughout this study.

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# ADJUSTMENT OF THE FLOW OF TISSUE FLUID IN THE PRESENCE OF LOCALIZED, SUSTAINED HIGH VENOUS PRESSURE AS FOUND WITH VARICES OF THE GREAT SAPHENOUS SYSTEM DURING WALKING

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(Received for publication April 21, 1937)

Varices of the saphenous systems of veins are a consequence of the inadequate valves present and the resulting imperfect circulation. Altered venous pressure relationships as one phase of this impaired circulation have heretofore received little attention. *The extremely abnormal pressure relationships found in varicose veins, appear only during activity.* The only method worked out for measuring the fluctuating venous pressure of exercise at the ankle, is that of Beecher, Field and Krogh (1). This method has been applied in the present study to a consideration of venous pressures during light and moderate exercise (walking) in patients with severe varices of the great saphenous system of veins. The pressure relationships found have been compared with those of normal subjects. The data obtained have been considered from the point of view of Starling's concept of the formation of tissue fluid and the formation of lymph.

The method for determining the fluctuating venous pressure has been described (1). Further experience with the method has made it possible to shorten appreciably the time of a determination. This is accomplished by minor changes of the apparatus and procedure. No change of principle has been found necessary. The revised method will be described briefly.

## APPARATUS AND METHOD

With each step, the venous pressure at the ankle fluctuates; this occurs as a consequence of weight bearing, or the squeezing of veins by muscles. To determine the venous pressure during walking, a maximum pressure ( $P_s$ ) and a minimum pressure ( $P_d$ ) must be determined during the step. These are analogous to systolic and diastolic pressures. It is also of interest to determine the pressure in the veins at rest immediately after the cessation of walking ( $P_o$ ), and the pressure after fifteen minutes of motionless standing ( $P_{15}$ ). In considering normal subjects it has been shown (1) that the degree of exer-

cise as well as the rate has an important effect on the venous pressure and on the pressure gradient from arterial to venous end of the capillary; accordingly, a standing (i.e. non-progressing) walk at two differing degrees of effort at the rate of forty steps per minute was used. Light exercise consisted in a standing walk at this rate, employing a low step with relaxed ankle, in which the ball of the foot was barely raised from the floor. Moderate exercise was obtained by a high step at the same rate; here, the foot was raised with relaxed ankle so that the toes were 10 cm. and the heel 25 cm. from the floor. A uniform step is very important; failure in uniformity, requires repetition of the experiment. Before each experiment the subject must take at least 10 steps to acquire uniformity and to allow for major adjustments of the deep circulation. Fatigue must be guarded against by frequent rest periods.

The modified apparatus is shown in Figures 1 and 2. A celluloid capsule 1.7 cm. wide and 0.7 cm. deep was sealed with celloidin to one of the prominent ankle veins, usually the prolongation of the great saphenous where it sweeps anterior to and just above the internal malleolus. A bony background, free from moving tendons, is best. The capsule (1) must be attached so well that at no time during an experiment will the edges separate and allow the flange of celloidin against the skin to act as a bellows with each step. If this occurs, the results are incorrect; consequently, the capsule must be examined before removal from the ankle to make certain this has not taken place. A flexible rubber tube of 1.5 mm. bore and 1 mm. wall thickness, 1.5 meters long was attached to the capsule. A short loop of this was caught against the leg with adhesive tape to take all tension off the capsule, and the other end attached to the proximal arm of a water manometer (2) made of glass tubing of 3.0 mm. bore. Each arm was 1.5 meters long. The manometer was filled with water to a height of 65 cm. This was fixed against a centimeter scale (3). A mercury reservoir (4) was arranged, as shown, in order that the air pressure in the system might be increased to any desired level. The distal end of the manometer was connected through rubber tubing 40 cm. long, and of the same dimensions as those given above, to a recording device (7). This recorder was made by cutting off a 3 cm. length of the barrel of a well fitting syringe and inserting in it a 1.7 cm. length of the piston. An air filled syringe (6) was inserted into the line connecting the water manometer with



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## APPARATUS AND METHOD

With each step, the venous pressure at the ankle fluctuates; this occurs as a consequence of weight bearing, or the squeezing of veins by muscles. To determine the venous pressure during walking, a maximum pressure ( $P_s$ ) and a minimum pressure ( $P_d$ ) must be determined during the step. These are analogous to systolic and diastolic pressures. It is also of interest to determine the pressure in the veins at rest immediately after the cessation of walking ( $P_o$ ), and the pressure after fifteen minutes of motionless standing ( $P_{15}$ ). In considering normal subjects it has been shown (1) that the degree of exer-

cise as well as the rate has an important effect on the venous pressure and on the pressure gradient from arterial to venous end of the capillary; accordingly, a standing (i.e. non-progressing) walk at two differing degrees of effort at the rate of forty steps per minute was used. Light exercise consisted in a standing walk at this rate, employing a low step with relaxed ankle, in which the ball of the foot was barely raised from the floor. Moderate exercise was obtained by a high step at the same rate; here, the foot was raised with relaxed ankle so that the toes were 10 cm. and the heel 25 cm. from the floor. A uniform step is very important; failure in uniformity, requires repetition of the experiment. Before each experiment the subject must take at least 10 steps to acquire uniformity and to allow for major adjustments of the deep circulation. Fatigue must be guarded against by frequent rest periods.

The modified apparatus is shown in Figures 1 and 2. A celluloid capsule 1.7 cm. wide and 0.7 cm. deep was sealed with celloidin to one of the prominent ankle veins, usually the prolongation of the great saphenous where it sweeps anterior to and just above the internal malleolus. A bony background, free from moving tendons, is best. The capsule (1) must be attached so well that at no time during an experiment will the edges separate and allow the flange of celloidin against the skin to act as a bellows with each step. If this occurs, the results are incorrect; consequently, the capsule must be examined before removal from the ankle to make certain this has not taken place. A flexible rubber tube of 1.5 mm. bore and 1 mm. wall thickness, 1.5 meters long was attached to the capsule. A short loop of this was caught against the leg with adhesive tape to take all tension off the capsule, and the other end attached to the proximal arm of a water manometer (2) made of glass tubing of 3.0 mm. bore. Each arm was 1.5 meters long. The manometer was filled with water to a height of 65 cm. This was fixed against a centimeter scale (3). A mercury reservoir (4) was arranged, as shown, in order that the air pressure in the system might be increased to any desired level. The distal end of the manometer was connected through rubber tubing 40 cm. long, and of the same dimensions as those given above, to a recording device (7). This recorder was made by cutting off a 3 cm. length of the barrel of a well fitting syringe and inserting in it a 1.7 cm. length of the piston. An air filled syringe (6) was inserted into the line connecting the water manometer with

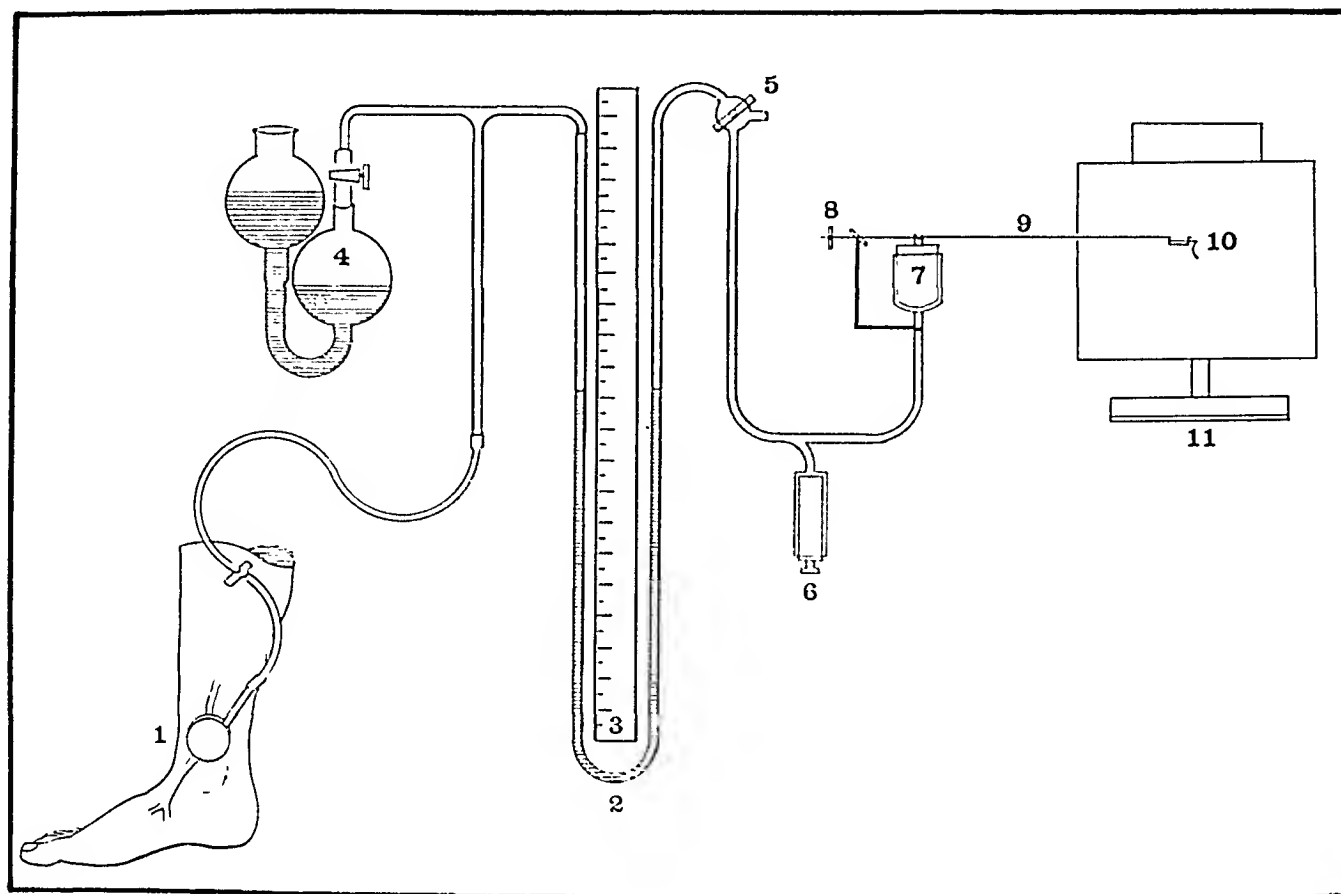


FIG. 1. APPARATUS FOR MEASURING VENOUS PRESSURE DURING WALKING

1, Capsule; 2, Manometer; 3, Scale; 4, Mercury reservoir; 5, Stopcock; 6, Air-filled syringe; 7, Piston recorder; 8, Counterbalance; 9, Hollow aluminum tube writing arm; 10, Steel wire writing point; 11, Kymograph.

the piston recorder; this was used to set the writing point, a hanging steel wire (10) at any desired level. The writing point was suspended from and freely movable in a plane perpendicular to a fine aluminum tube (9), about 1.0 mm. in diameter. This must be attached to the piston of the syringe and then carefully counterbalanced (8).

The maximum pressure ( $P_s$ ) can be determined in two ways. Previous work has shown that the two methods agree excellently; therefore only one (the direct) was employed in this study. Occasionally it was confirmed by the indirect. First, it is possible to measure  $P_s$  by direct observation. One observer watches the vein through the capsule (cross lighting increases the accuracy here) while another elevates the pressure in the system by raising the mercury reservoir. Finally, a point is reached as the pressure rises, where the blood flows only during the moment the tensed foot and leg are bearing all the subject's weight. The moment of maximum pressure occurs just before the foot is raised. When the foot is elevated, the vein blanches. When near the endpoint a further increase of intracapsular pressure of 1 to 3 cm.  $H_2O$  prevents the flow even during the moment of weight bearing, i.e., the moment of maximum venous pressure. This point can be checked easily by the same or another individual to within 3 cm. of water.

$P_s$  can be determined by a second, and in this case objective, method. If a high pressure is introduced into the system and then gradually lowered while the subject walks, it should be possible to find  $P_s$  by observing the pressure at which waves begin to come through on the kymograph (11). Practically, one must accept the fact that however carefully the location of the capsule is chosen, there will be some motion of the underlying tissues with each step, some change in the intracapsular pressure which is not due to blood flow. This will be reflected on the kymograph as a curve of constant shape and amplitude. Now, if the pressure is gradually lowered, a point will be reached where the shape and amplitude of the curve shows a sharp change, due to the onset of blood flow through the vein imprisoned by the capsule. This represents  $P_s$  and agrees very well with it as determined by the direct method.

To determine the minimum pressure ( $P_d$ ) in the vein during the step, one starts with a relatively high pressure, say near  $P_s$ , and gradually lowers it. As the pressure is lowered the volume of the fluctuations of the vein underlying the capsule increases and is recorded as a curve of greater amplitude on the kymograph. Finally, this passes through a maximum and begins to decrease even though the intracapsular pressure continues to fall. By obtaining short sections of curve, about 25 steps long, for



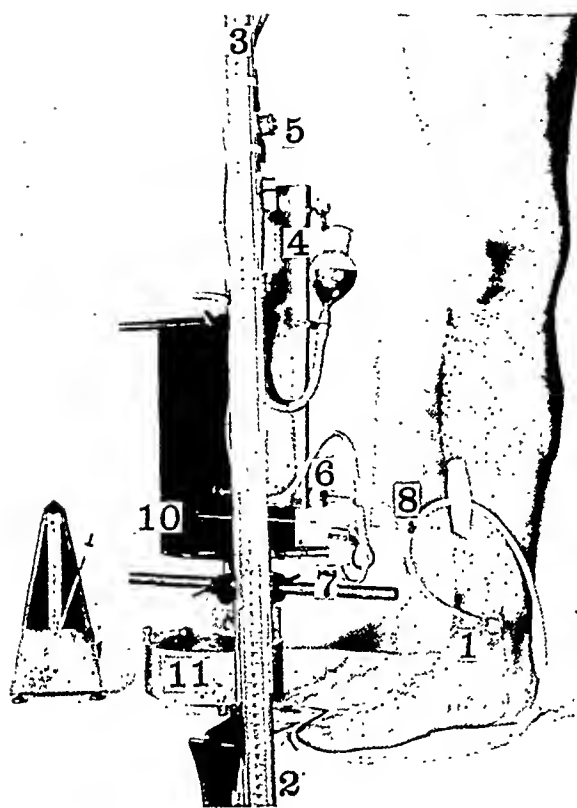


FIG. 2. APPARATUS FOR MEASURING VENOUS PRESSURE DURING WALKING

1, Capsule; 2, Manometer; 3, Scale; 4, Mercury reservoir; 5, Stopcock; 6, Air-filled syringe; 7, Piston recorder; 8, Counterbalance; 10, Steel wire writing point; 11, Kymograph.

each 5 cm.  $H_2O$  the pressure is lowered in the system; this point can be demonstrated sharply in a normal individual. It is convenient to have the kymograph going at slow speed so that the tracing of 25 steps will be condensed into about 1.5 cm. length. If, between each section of tracing, stopcock 5 is opened to the atmosphere while the pressure in the system is readjusted, the writing point will remain at a constant level and the time of adjusting this will be saved. The pressure at which the maximum fluctuation of the curve occurs represents the minimum venous pressure, for at this point the maximum filling and emptying of the vein occurs during the step. If the intracapsular pressure be increased above this point the vein cannot fill completely, and the amplitude of fluctuation is less; a smaller fluctuation also occurs if the intracapsular pressure be lowered below the critical point. In this case, the smaller fluctuation is due to the fact that the vein does not empty completely; so the critical point (at which maximum filling and emptying of the section of vein imprisoned by the capsule occur) represents the minimum pressure in the vein during walking. In normal subjects this can be determined to within 5 cm.

$H_2O$  pressure, and usually within 3 cm. In cases with varicose veins, in which the venous pressure fluctuation is lacking, there will be during the course of the experiment, a gradual alteration in the curve due to changing pressure within the capsule; but in these cases even a slight change in amplitude is spread out over a wide pressure range and has no similarity to the sharp end-point which is present when a truly fluctuating venous pressure exists.

With a little practice  $P_0$  can be determined one second after the cessation of walking. The pressure in the ankle veins after the patient had been standing motionless equally on both feet for 15 minutes,  $P_{15}$ , was obtained without backboard support. This is of use in studying the effect of "involuntary" muscular activity.

Pertinent data regarding the normal individuals and those with varicose veins are listed in Tables I and II. The subjects were loosely clothed. Studies were started only after a 30 minute preliminary rest period. All determinations were made on barefoot subjects, and, in those with varicose veins, on the ones in whom valves of the great saphenous system could not be demonstrated clinically and in whom gross edema was not present. This choice was made since clinical edema in uncomplicated cases of varices is the exception. The rate and quantity of edema formation in the dependent legs of subjects sitting for 2 hours has been measured. Normals have been compared with uncomplicated cases with varicose veins. The edema formation is essentially the same in both groups, unless the perforating veins are incompetent.

#### DISCUSSION

The data in Table I indicate that in normal subjects a fluctuating pressure occurs in the great saphenous system of veins at the ankle. During light exercise this rises to 75 cm.  $H_2O$ , well above the colloid osmotic pressure of the blood (40 cm.  $H_2O$ ), but persists at this high level for only one-third of the time required for the step (2). During two-thirds of the time required for the step the pressure of 28 cm.  $H_2O$  is well below the colloid osmotic pressure of the blood, and the conditions are suitable for the absorption of tissue fluid. With increasing severity of exercise, the pressures are even more favorable for absorption of tissue fluid directly into the venous end of the capillaries; any fluid not so absorbed is carried off by the lymphatics. These points have been discussed elsewhere (2) and are recalled here in order to emphasize the differences encountered when dealing with varicosities of the saphenous system. (See Table II.) The most striking abnormality is the persistently high and non-fluctuating pressure in the veins. During light exercise it persists at 96 cm.  $H_2O$ . The sus-

TABLE 1\*

*Normal subjects*(Pressures in cm. H<sub>2</sub>O at ankle level. Average height of heart to mid capsule 125 cm.)

Subject	Sex	Light exercise (Walking)				Moderate exercise (Walking)				Resting pressure, 15 minute standing $P_{15}$
		Maximum pressure $P_s$	Minimum pressure $P_d$	Resting pressure 0 time $P_0$	Pulsation pressure $P_s - P_d$	Maximum pressure $P_s$	Minimum pressure $P_d$	Resting pressure 0 time $P_0$	Pulsation pressure $P_s - P_d$	
H. B.....	♂	69	30	62	39	41	21	40	18	111
		72	30	54	42	46	21	42	22	119
M. N.....	♂	100	34	67	66	62	21	45	39	120
		108	26	71	82	69	20	52	49	
C. T.....	♂	51	15	45	36	46	10	41	36	95
		59	10	48	49	50	9	39	42	
O. B.....	♂	105	30	96	75	66	18	63	46	
		107	35	81	72	62	24	52	38	
A. K.....	♂	69	20	63	49	72	21	60	52	106
G. B. H.....	♀	72	33	58	39	55	25	44	28	102
		61	36	57	25	51	25	48	28	
M. F.....	♀	59	24	54	35	45	18	43	27	108
		63	27	56	36	53	15	48	36	108
M. B.....	♀	84	25	71	59	49	17	38	35	109
		71	22	64	49	53	18	40	33	
J. M.....	♀	85	50	75	35	68	34	63	34	
		86	47	76	39	66	32	65	35	
B. K.....	♀	62	17	54	45	62	14	63	46	
		69	18	49	51	64	11	53	49	
Average (to nearest unit)...		75	28	63	49	57	20	49	36	109

\* Adapted from Beecher, Field and Krogh (1).

tained pressure in the varicosities is more than twice the normal colloid osmotic pressure of the blood and a gross positive filtration pressure of the order of 50 cm. H<sub>2</sub>O is evident, yet *in the cases studied gross edema was not present.*

It was shown (loc. cit.) that with increasing exercise a more efficient circulation was provided for by a marked steepening of the pressure gradient from the arteriolar to the venous end of the capillary. With varicosities this is lacking insofar as it is a result of lowered venous pressure. With increasing exercise a tremendous increase in washing of the tissues with reabsorption of much of the tissue fluid directly into the capillary is provided for normally (2, 5, 6, 7, 10).

*With varicosities a sustained high filtration pressure exists; thus the great burden of removing tissue fluid must fall on the lymphatics.* The lymphatics appear to provide the compensating mechanism which permits such high filtration

pressures to exist without gross edema. It is probable that in the failure of this compensating mechanism some explanation of the severe complications of varices can be found.

It will be useful to consider the data from the viewpoint of Starling's theory (11) of the formation of tissue fluid. In its simplest form this theory considers that the movement of fluid across the capillary wall is the resultant of two opposing forces, the colloid osmotic pressure of the plasma tending to draw fluid into the vessel and the hydrostatic pressure tending to force it out. This concept holds true if one remembers that the effective pressures are the net pressures between the inside and the outside of the capillary and if one regards what Peters (9) calls the "subtle implications" of the theory: that the end results may be influenced by variable capillary permeability, by tissue elasticity and by lymph flow. The reasons why all three of these are of importance

in the case under consideration will be pointed out.

In the tissues drained by the superficial system of leg veins a condition exists which is comparable in some respects to that found in the presence of prolonged and marked venous obstruction. Landis (6) has shown that venous obstruction is followed by elevation of arteriolar capillary pressure to a height greater than that producing the constriction, in the case of a recumbent subject with hand at the level of the manubrium sterni. Thompson, Thompson and Dailey (12) have shown that standing still results in the escape of what they describe as approximately protein-free fluid from the capillaries to the leg tissue with increase in leg size. Youmans et al. (14) have confirmed the increase of the size of the legs with standing. Landis et al. (8) have shown that when the venous pressure exceeded 60 mm. Hg (81 cm. H<sub>2</sub>O), protein in an appreciable quantity escaped. At a venous pressure of 80 mm. Hg (108 cm. H<sub>2</sub>O), protein was lost in an amount which indicated that the capillary filtrate contained an aver-

age of 1.5 per cent protein. This, of course, would facilitate the loss of fluid from the blood and would tend to increase the gross filtration pressure of 50 cm. H<sub>2</sub>O referred to above, since it would act outside the capillary. von Farkas (3) and Govaerts (4) estimate that 1 per cent serum albumin produces an osmotic pressure of 6 to 8 cm. H<sub>2</sub>O.

In brief, important forces or conditions tending to produce tissue fluid here are hydrostatic pressure (gravity tends to produce filtration at the arterial end and to prevent reabsorption at the venous end of the capillary) and the consequences of increased capillary permeability. Those tending to oppose the production of tissue fluid are concerned chiefly with increasing the colloid osmotic pressure of the blood. Such increase is probably of little effect. It can be explained as a result of plasma concentration due to accelerated filtration and to acidification of the blood due to the loss of oxygen and accumulation of carbon dioxide causing the red blood cells to withdraw fluid from the plasma, as pointed out by Peters.

TABLE II  
*Subjects with varicose veins*  
(Pressures in cm. H<sub>2</sub>O at ankle level)

Subject	Sex	Height of heart to mid-capsule	Light exercise (Walking)				Moderate exercise (Walking)				Resting pressure, 15 minute standing $P_{15}$
			Maximum pressure $P_s$	Minimum pressure $P_d$	Resting pressure 0 time $P_o$	Pulsa-tion pressure $P_s - P_d$	Maximum pressure $P_s$	Minimum pressure $P_d$	Resting pressure 0 time $P_o$	Pulsa-tion pressure $P_s - P_d$	
J. B. ....	♂	cm. 119	110 113	Same as maximum pressure	93 93	0	93 101	Same as maximum pressure	80 82	0	109 110
M. M. ....	♂	120	83 89	Same as maximum pressure	81 85	0	77 85	Same as maximum pressure	77 81	0	93 99
C. G. ....	♂	127	114 117	Same as maximum pressure	104 104	0	110 119	Same as maximum pressure	107 113	0	109
T. M. ....	♂	127	91 91	Same as maximum pressure	91 93	0	91 93	Same as maximum pressure	93 89	0	113
G. McC. ....	♂	126	92 91	Same as maximum pressure	83 86	0	80 76	Same as maximum pressure	78 76	0	
D. S. ....	♂	132	90 94	Same as maximum pressure	90 96	0	72 64	Same as maximum pressure	76 70	0	108 106
N. W. ....	♀	110	101 105	Same as maximum pressure	83 89	0	94 97	Same as maximum pressure	89 94	0	85 89
M. A. ....	♀	111	79 79	Same as maximum pressure	81 85	0	85 89	Same as maximum pressure	85 87	0	85 87
D. M. ....	♀	105	58 60	Same as maximum pressure	60 60	0	58 56	Same as maximum pressure	54 52	0	67
M. H. ....	♀	117	129 129	Same as maximum pressure	122 124	0	115 115	Same as maximum pressure	110 114	0	110 110
Average. ....		119	96	96	90	0	89	89	85	0	99

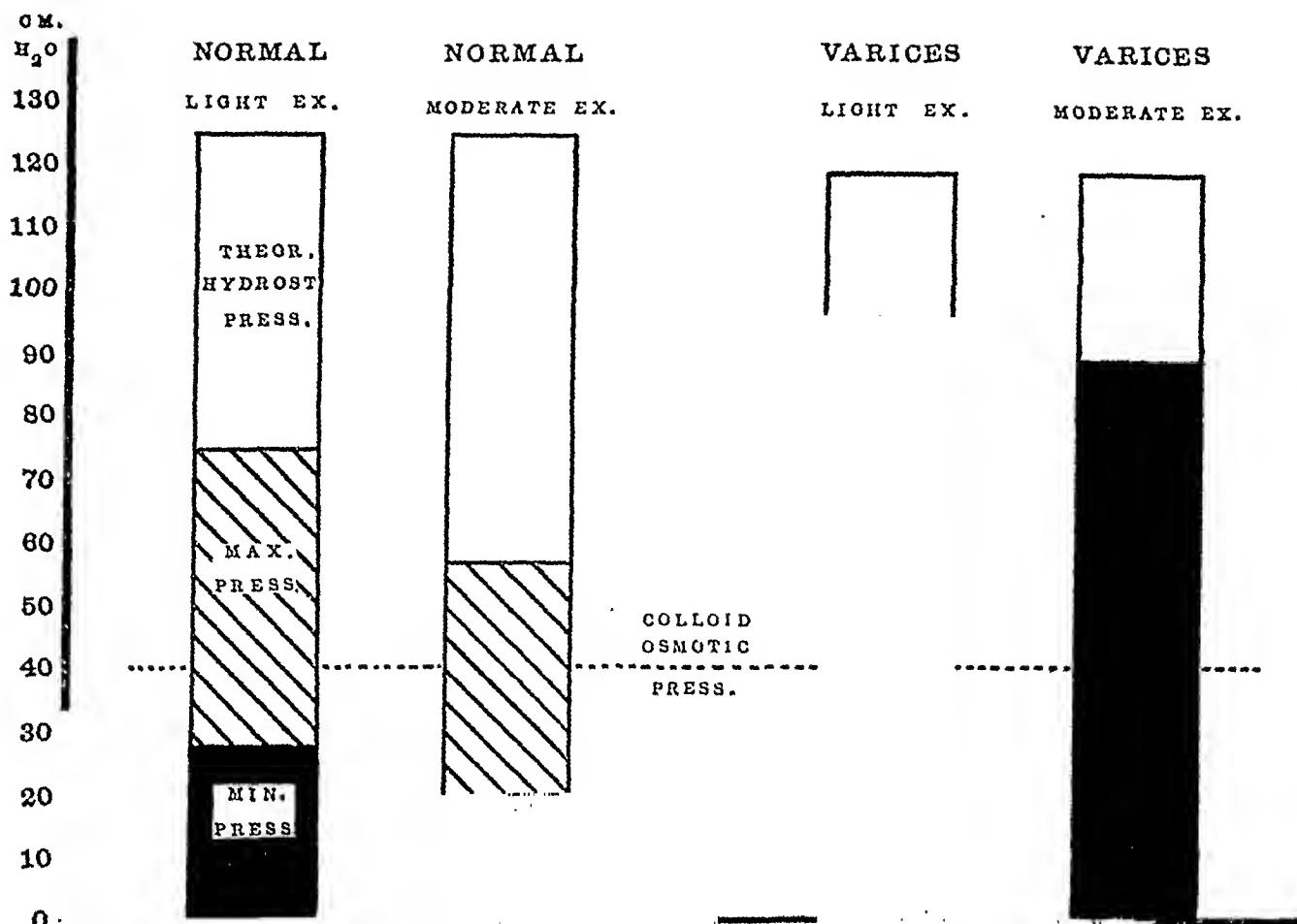


FIG. 3. PRESSURE RELATIONSHIPS AT THE ANKLE LEVEL DURING WALKING, IN NORMAL AND VARICOSE VEINS

The difference between the maximum and minimum pressures represents the pulsation pressure with each step; it is lacking in the varicose veins.

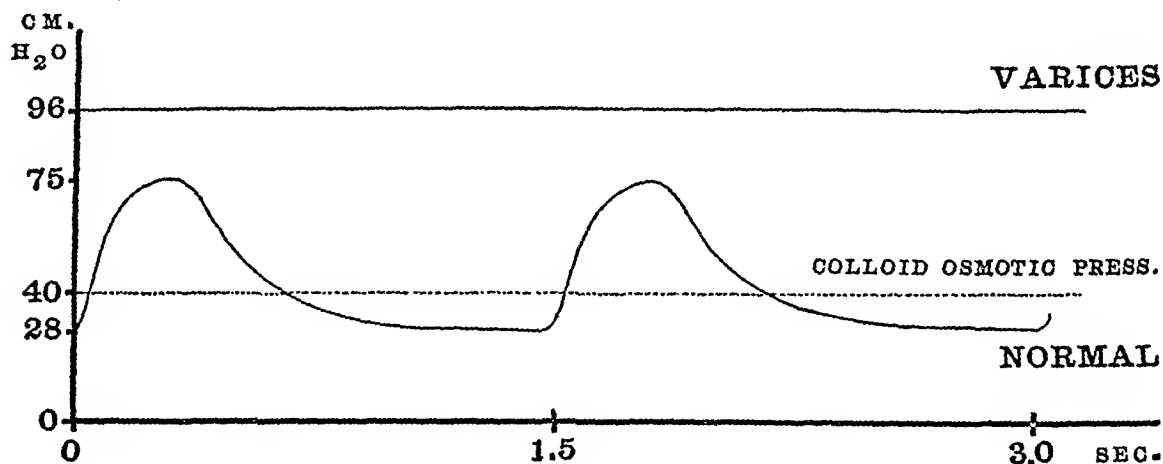


FIG. 4. SHOWS THE PRESSURE FLUCTUATION IN NORMAL ANKLE VEINS DURING TWO STEPS

Normally for two-thirds of the time of the step the pressure is below the colloid osmotic pressure of the blood. The sustained, high venous pressure found in varices during walking is shown.

Both of these effects would be increased by the venous stasis present. Normal tissue elasticity would act in opposition to filtration of fluid from the capillaries. Presumably, as soon as the "tissue spaces" were filled with fluid, the elasticity of the walls of the spaces would oppose further filtration. This is supported by Waterfield (13), who found that changes in volume during standing in subjects in poor training, with fat, flabby legs, showed greater increases than was the case with subjects who were in training and who had tightly knit calves and ankles. Krogh, Landis and Turner (5) found that when tissue fluid had accumulated to the extent of more than 0.6 cc. per 100 cc. of arm tissue, venous obstruction of 15, 20 and at times 30 cm.  $H_2O$  failed to increase further the accumulation. Probably at this point of "saturation," removal through lymphatics begins.

Tissue tension plus accelerated lymph flow must balance the action of the effective filtration pressure in the subjects studied, for gross edema did not appear. The explanation of this balance as due in any appreciable degree to a high tissue tension, in the absence of gross edema, is untenable for physical reasons. Since reabsorption into the capillaries is impossible because of the high pressures in them, the balance must be maintained by an increased lymph flow. It is evident from this data that a high, sustained venous pressure of the degree indicated above is not of itself adequate for the production of gross edema.

#### SUMMARY

Venous pressures at the ankle level have been measured during walking in subjects having incompetent valves of the great saphenous system. Gross edema was not present in the subjects studied. A gross filtration pressure of the order of 50 cm.  $H_2O$  in excess of the colloid osmotic pressure of the blood was shown to be present even during walking. The conditions present are in some respects comparable to those found in the presence of a prolonged partial venous obstruction. The normal reabsorption of tissue fluid at the venous end of the capillary as postulated and supported by many investigators is impossible here, and all of the tissue fluid must be carried off by the lymphatics. It is pointed out that failure

of this compensating mechanism may be responsible for some of the severe complications of varicose veins. The data are discussed from the point of view of Starling's theory of the formation of tissue fluid.

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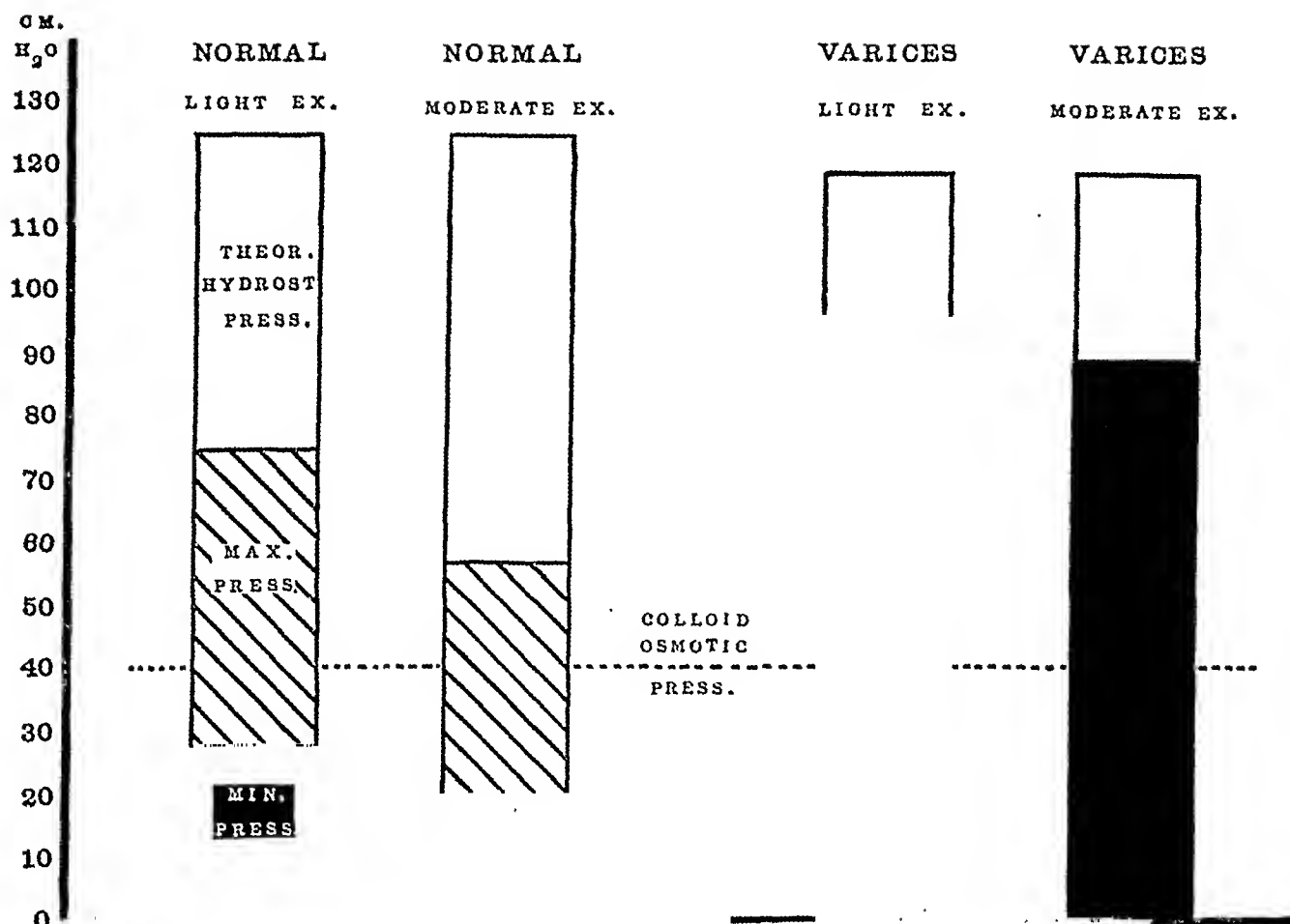


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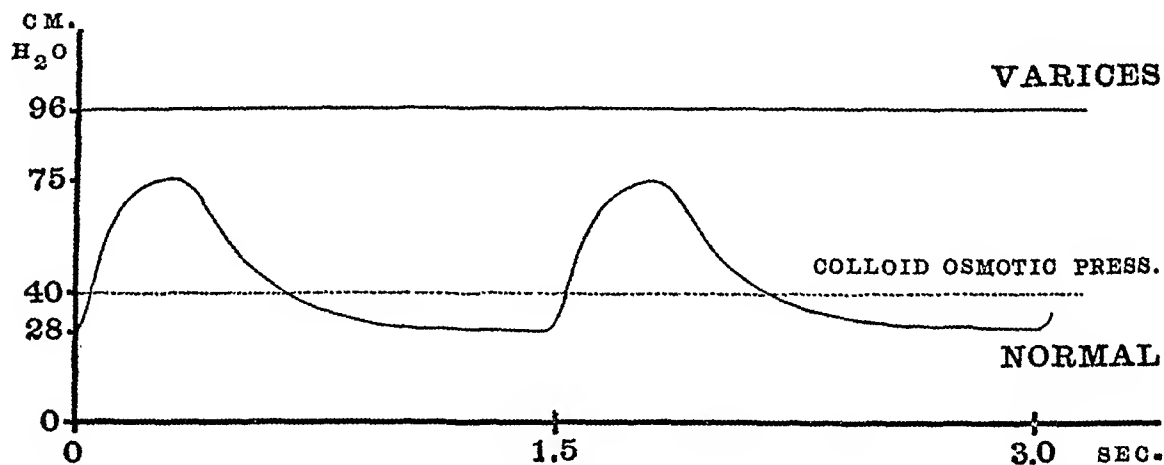


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# THE COAGULATION DEFECT IN HEMOPHILIA. THE EFFECT IN HEMOPHILIA OF INTRAMUSCULAR ADMINISTRATION OF A GLOBULIN SUBSTANCE DERIVED FROM NORMAL HUMAN PLASMA<sup>1</sup>

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The recent investigations of Patek and Stetson (1)<sup>2</sup> indicate that the defect in coagulation of blood in hemophilia resides in the plasma rather than in the platelets. Patek and Taylor (2, 3)<sup>2</sup> isolated a substance from citrated normal cellular-free plasma, by isoelectric precipitation, with which was associated the clot-promoting factor of normal plasma. This substance was effective, both in vitro and after intravenous injection, in reducing the coagulation time of hemophilic blood. By the same method, a similar substance in approximately the same quantity was obtained from citrated hemophilic plasma, but its clot-accelerating power on hemophilic blood was much less marked. In the absence of definite knowledge concerning the nature of the effective material it was called "globulin substance" which is the nomenclature that will be retained in this communication. The material was thermolabile, insoluble in water, partially soluble in physiological saline solution, and non-diffusible. It was precipitated in optimal amounts between pH 5.9 and 6.4. The material passed readily through a Berkefeld filter without loss of potency and was almost completely inactivated by small excesses of alkali (3). Bendien and Creveld (4) have isolated a substance, by dilution and acidification, from normal serum which they injected intramuscularly and intravenously and report favorable results.

Patek and Taylor's (3) studies imply that in some respects hemophilia is a deficiency disease in which certain factors present in normal cellular-free plasma are either reduced or modified. The present communication reports a study of the effects of intramuscular administration of

globulin substance on the blood coagulation time of hemophilic subjects, and on the content of a clot-promoting factor in the blood following such administration.

## METHODS

*Coagulation time.* In general the standard procedure followed was the same as that used in previous studies (1, 3) made in this laboratory. All coagulation times were determined on venous blood. Hypodermic syringes and number 20 steel needles for venepuncture were freely rinsed with physiological saline solution immediately before use. The blood was taken under stasis from arm veins, the tourniquet being removed after the sample was withdrawn. If venepuncture was not immediately successful, another vein was used and another syringe and needle employed. Two cc. of blood so drawn were transferred, after removal of the needle from the syringe, with minimum agitation, to 100 × 13 mm. test tubes having an inside diameter of 11.5 to 12 mm. The tubes were cleansed with concentrated bichromate-sulphuric acid solution and thoroughly rinsed free from this material with distilled water and finally with physiological saline solution. The presence of air bubbles in the blood sample was avoided because they tend to shorten the coagulation time.

When test substances were to be assayed they were pipetted into the test tubes prior to the addition of the blood. Further mixing was not found necessary.

Immediately after the addition of whole blood, duplicate tubes were placed in a water bath at 37.5° C. Agitation of the tubes was avoided. Only one of the duplicate tubes was read from time to time by gently tilting until just before the end point, when both tubes were read. The coagulation time was the interval elapsing from withdrawal of blood to the time when the tubes could be slowly inverted without loss of contents. The times for the two tubes usually checked very closely, and the average time was taken as the true coagulation time. When there was a considerable discrepancy, the longer coagulation time only was recorded. Normal controls by this method, independent of sex, gave values for the coagulation time of venous blood of from 6 to 12 minutes.

*Control period.* The investigation was carried out on seven hemophilic patients between the ages of 16 and 47 years who had been under observation in this clinic for

<sup>1</sup> The expenses of this research were defrayed in part by a gift to Harvard University from Smith, Kline and French Laboratories of Philadelphia.

<sup>2</sup> Review of literature here.

isotonic saline solution. After centrifuging and Berkefeld filtration, 65 cc. were available for injection. This standard test dose represented about one-third of the dosage previously given intravenously (3).

Four hemophilic patients with coagulation times between 35 and 85 minutes were injected intramuscularly with a single standard test dose of globulin substance. Figure 1 shows the results obtained in a case in which the coagulation time

time in all cases similar to that recorded in Figure 1. However, when the *second* injection was given at any time within a 7-hour period there was not more than a slight further decrease in the coagulation time. A typical response is shown in Figure 2 (I. M.). In one instance a second injection failed to reduce the coagulation time to the point attained by the initial injection.

A hemophilic patient was injected with a standard test dose of globulin substance by the intra-

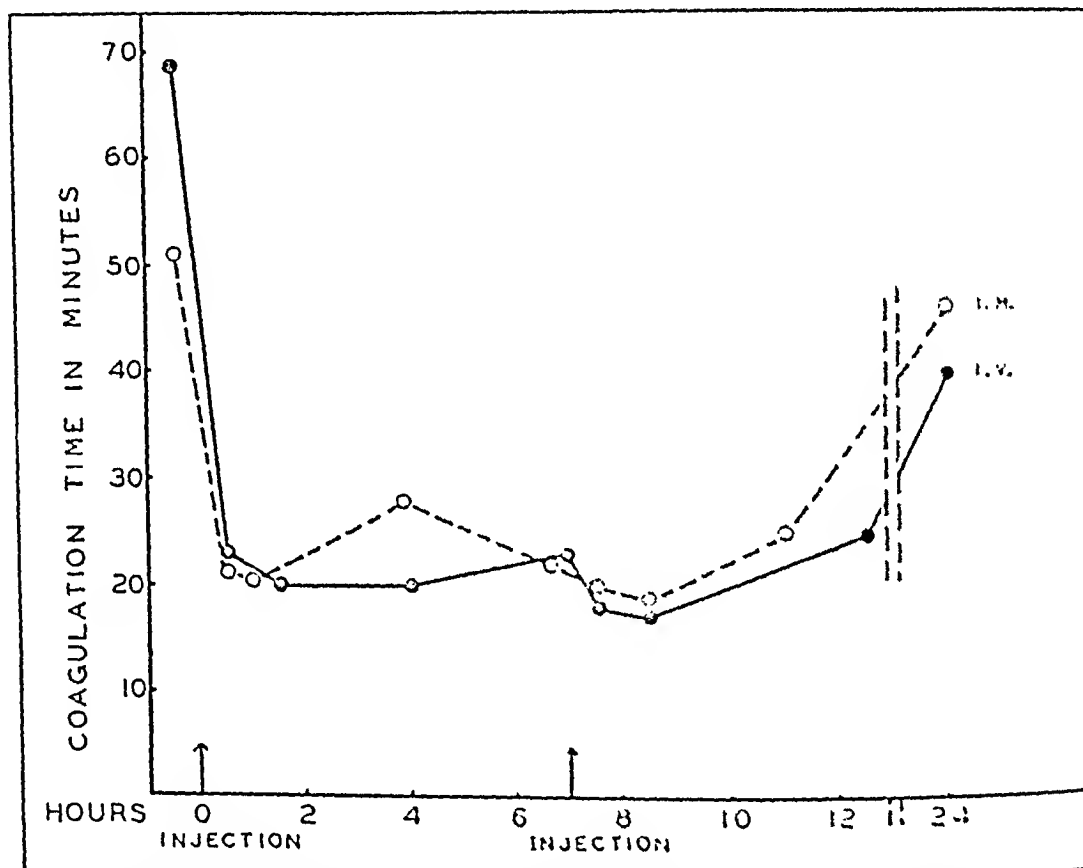


FIG. 2. EFFECT OF REPEATED INTRAMUSCULAR AND INTRAVENOUS INJECTIONS OF "GLOBULIN SUBSTANCE" ON COAGULATION TIME OF HEMOPHILIC BLOOD

was followed at 4-hour intervals for a 24-hour period after injection. Comparable data were obtained on the remaining cases. There was a favorable response in all instances. There was a sharp fall in the coagulation times to minimum values in from 30 minutes to 1 hour after injection, which were sustained for several hours. The coagulation times returned to pre-injection levels in 24 hours.

*The effect of repeated parenteral injections of globulin substance.* Two intramuscular injections of a standard test dose of globulin substance were given to five subjects. Following the *initial* injection there was a prompt drop in the coagulation

venous route. The results are plotted in Figure 2 (I. V.). There was an immediate abrupt fall in the coagulation time. A slight further drop followed a second intravenous injection 7 hours later. It may be observed (Figure 2) that the results obtained by the use of the intramuscular route are entirely similar to those obtained after intravenous injections.

Observations were made on one hemophilic subject to determine the effects of repeated intramuscular injections of standard test doses of globulin substance over a 24-hour period. Injections were given at 6-hour intervals. Following the *initial* injection there was the usual prompt

fall in the coagulation time (Figure 3). The *second* injection reduced the coagulation time to the level obtained by the initial injection. However, the *third* injection failed to reduce the coagulation time to the point attained by either of the first two injections. The *fourth* injection did not effect the coagulation time which continued to rise to the pre-injection level.

It is known from three sets of observations that if a hemophilic subject is re-injected either intramuscularly or intravenously with globulin substance 24 hours after the last injection, that the entire cycle of effect on his coagulation time repeats itself.

by observing its effect on a second hemophilic blood *in vitro*. Immediately before and 1 hour after each injection of the standard test dose of globulin substance, blood was drawn and citrated to a final concentration of 0.25 per cent sodium citrate. In each instance 0.1 cc. of the whole citrated blood was mixed with 2 cc. of blood from a second control hemophilic patient. This control patient had a coagulation time of 127 minutes which did not vary significantly during the period of observation. The results are given in Figure 4. This figure shows graphically the coagulation time of the control patient's blood after adding citrated whole blood obtained from the injected

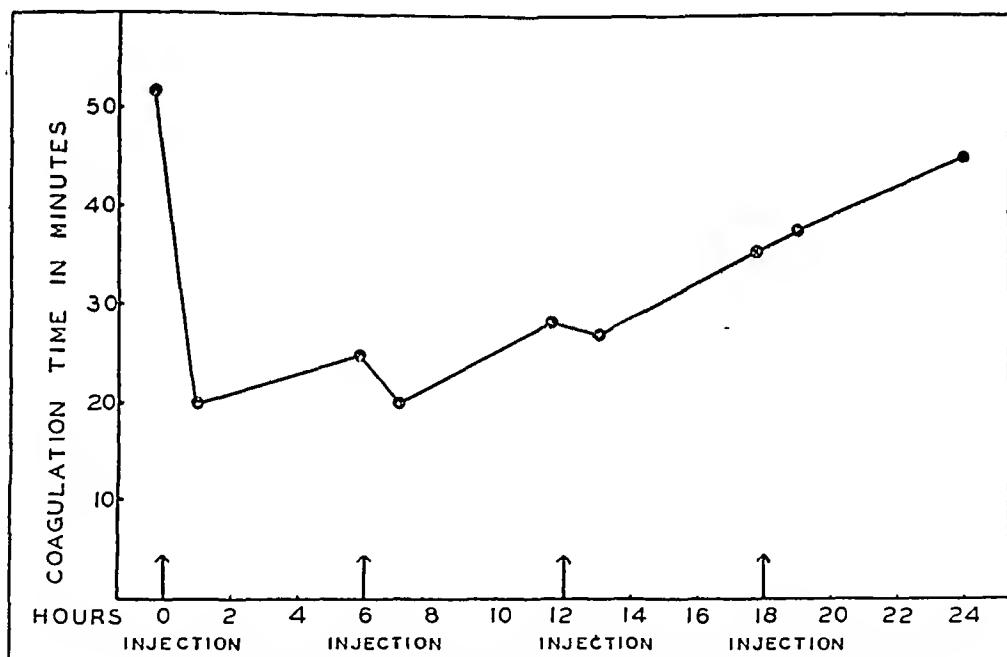


FIG. 3. EFFECT OF MULTIPLE INTRAMUSCULAR INJECTIONS OF "GLOBULIN SUBSTANCE" ON COAGULATION TIME OF HEMOPHILIC BLOOD

*Changes in the clot-promoting power of hemophilic blood and plasma following the intramuscular injection of globulin substance.* Because of the evidence that frequently repeated injections of globulin substance had a diminishing effect in reducing the coagulation time of hemophilic blood, it became desirable to attempt to determine the amount of clot-promoting factor in the circulating blood under these circumstances.

An approximation of the coagulant activity of the whole blood in the subject receiving repeated intramuscular injections (Figure 3) was obtained

patient. In other words, it may be considered that the data indicate the changes in concentration of clot-promoting factor present in the injected patient's blood. Figure 4 shows that the clot-accelerating power of the blood was increased after injections of globulin substance. In comparing Figure 3 with Figure 4 the important conclusions are that the coagulation time has risen in spite of repeated injections, while the concentration of clot-promoting material has increased in the patient's blood.

Since relatively large amounts of blood are re-

quired to prepare globulin substance, the actual preparation of the material from the patient every few hours is not feasible. However, it has been shown by dialysis experiments that the coagulant activity of plasma resides in the globulin substance

philic plasma possessed a slight clot-accelerating power when added to another hemophilic blood. However, it was not possible to demonstrate that the severity of the case as judged by the length of the coagulation time, could be correlated with

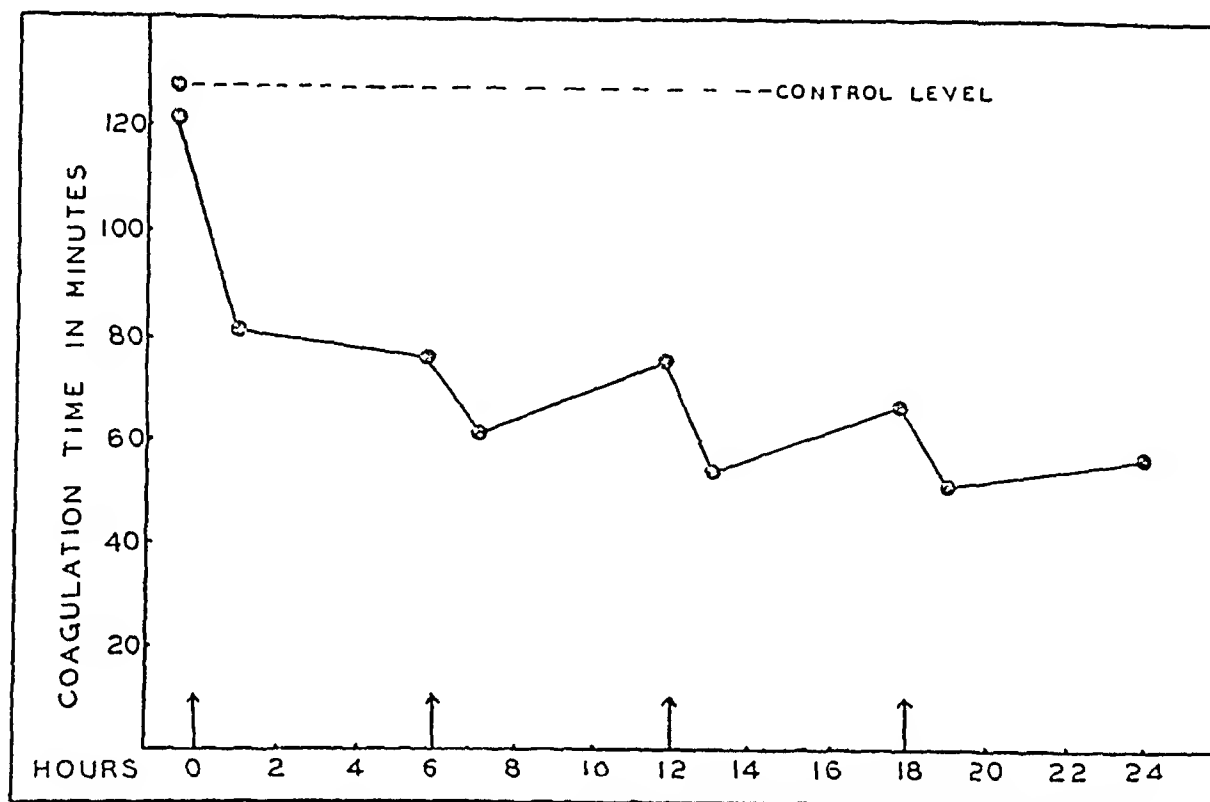


FIG. 4. EFFECT OF ADDING WHOLE BLOOD FROM A HEMOPHILIC SUBJECT INJECTED WITH "GLOBULIN SUBSTANCE" AT TIMES INDICATED, ON COAGULATION TIME OF CONTROL HEMOPHILIC BLOOD *IN VITRO*

(3). Hence the clot-promoting activity of plasma is an indirect measure of globulin substance. By the use of a similar technique of titration against a control hemophilic blood *in vitro* mentioned above, the increase in concentration of the clot-promoting factor was demonstrated in the cellular-free plasma following the intramuscular injection of globulin substance in three additional cases of hemophilia. In each instance the rise in coagulation time of the injected patient's blood, following the favorable response, occurred before the concentration of the coagulant factor in the plasma reached its maximum.

#### SUMMARY AND DISCUSSION

The quantitative nature of the action of normal globulin substance on hemophilic blood *in vitro* has been indicated (3) and is confirmed by the studies presented here. In all instances hemo-

the degree of the clot-promoting activity of the plasma.

When a suspension of globulin substance derived from 300 mgm. of dried material was injected intramuscularly in hemophilia, the response was a sharp drop in the coagulation time which was sustained at low levels for approximately 8 hours and returned to the pre-injection level within 24 hours. The effectiveness of globulin substance is not increased by intravenous administration.

The observations with repeated intramuscular or intravenous injections of globulin substance strongly indicates that there is a refractory phase for repeated injections. It appears that during such a phase, the coagulation time of the blood in hemophilia increases although the concentration of globulin substance in the circulating plasma is not diminished. The refractory period is not

longer than 24 hours since an injection at that time again gives the optimal effect.

The refractory period cannot be associated with tissue fixation of the injected material since similar effects are experienced when second injections are made intravenously. Nor is there failure of absorption as proven by the method described for measuring the concentration of clot-promoting factor in the blood stream. The time factor would indicate that the phenomenon is not one of an antigen-antibody reaction. The actual cause of the refractory period and its nature awaits further investigation.

Mellanby (5) showed that the slow injection of small quantities of certain clot-accelerating snake venoms in animals produced a non-coagulable period. Mills (6) gave repeated small injections of tissue extracts to animals and observed a negative phase. These investigators ascribed the non-coagulable phase to defibrination of the blood. Eley, Green and McKhann (7) working with placental extract report no non-coagulable period in animals with the dose employed although they quote Sakurai as having noted such a phenomenon. However, there is no evidence in our data to indicate that a non-coagulable phase follows the administration of globulin substance in hemophilia. In no instance did the coagulation time of the blood rise above the initial level.

#### CONCLUSIONS

1. Globulin substance prepared from normal human plasma accelerates clot-formation of hemophilic and normal blood *in vitro* in a quantitative manner.

2. Globulin substance may be administered intramuscularly in hemophilia with reduction of the

coagulation time similar to that produced by its intravenous injection.

3. Following the *initial* injection of globulin substance in hemophilic subjects, a refractory phase is established. During this period the coagulation time is little affected by subsequent injections although it has been shown that the concentration of the clot-accelerating material is progressively increased in the circulating blood of the injected patient. Recovery from this state is complete in 24 hours.

4. There is no evidence that a non-coagulable phase occurs following the administration of globulin substance.

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# THE OPSONO-CYTOPHAGIC TEST IN CHILDREN WITH PERTUSSIS AND IN CHILDREN VACCINATED WITH *H. PERTUSSIS* ANTIGENS<sup>1</sup>

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The mechanism of immunity in pertussis is not known. It would be easy to say, but rather difficult to prove, that the immunity resides in the fixed tissue cells of the respiratory tract. There is no clinical test for immunity other than intimate exposure to the disease itself. Intimate exposure is reported to produce the disease in 70 to 80 per cent of children with negative histories (1). It still remains to be proven that it is possible to immunize actively against pertussis; the possibility seems likely, however, since the introduction of vaccines made from recently isolated, toxic strains having the characteristics of the "Phase 1" strains described by Leslie and Gardner (2). In three field studies where such strains were used favorable results were reported (1, 3, 4). In another very thorough study (5) in which the method of preparation of vaccine was slightly different unequivocal evidence of immunization was not obtained. It is obvious that a convenient clinical test of immunity would greatly facilitate the trial of immunizing agents.

The complement fixation test and the agglutination reaction are primarily of academic interest though in Denmark the former is employed as a diagnostic procedure in cases of persistent bronchitis. These methods of detecting circulating antibody usually fail, three to five months after the antigenic stimulation, whether it be infection or vaccine. Curves of complement fixing antibody titers following vaccination (6, 7, 8, 9) are similar to those obtained after the disease (10, 11, 12, 13). Recently Mishulow and coworkers (14) have compared the curve of agglutinin titers after vaccination with that during and after an attack of the disease. Higher titers were obtained and persisted longer after an attack. These data are of great interest in that they prove that the vaccine employed stimulates the formation of *circulating*

*antibody*. In the individual who has recovered from pertussis, we know, from clinical observation, that the failure to detect circulating antibody by these tests several months after recovery does not mean an absence of immunity. Do the immune bodies actually disappear from the blood and localize in the tissues, or are our methods of detecting them in the blood inadequate? When the complement fixation and agglutination reactions again fail us two or three months after vaccination, how can we test for immunity? There are two lines of approach left open. We can assume that antibody has left the blood and attempt to test for it in the tissues. The skin, at least, is available. Skin reactions due to the allergy of infection have been reported and also skin reactions of the Schick type.

The claims of the existence of specific skin hypersensitivity during and after an attack of pertussis (15 to 22) are undoubtedly plausible despite reports to the contrary (23, 24, 25). Specific allergic skin reactions occur in many bacterial infections. Because a positive allergic reaction occurs after an attack of pertussis, it is assumed to indicate immunity. A negative reaction is assumed to indicate susceptibility. Here one enters the controversy of the relation of allergy to immunity which is not well understood.

The description by Siebler and Okrent (26) of a Schick type of reaction, positive before the disease and negative afterwards, is difficult to credit. No circulating anti-endotoxin for *H. pertussis* has been demonstrated in man. *H. pertussis* vaccination of animals has failed to protect the skin from the necrotic action of *H. pertussis* endotoxin (27, 28).

A satisfactory skin test for immunity may, in the future, be developed using the allergic type of reactions mentioned above. However, it seems worth while to follow the other lead left open. Does the failure of detection of circulating anti-

<sup>1</sup> Aided by a grant from the Christine Breon Fund.

body by the complement fixation and agglutination reactions a few months after recovery prove its absence? The recent report of Kendrick, Gibbs and Sprick (29) utilizing the opsono-cytophagic test indicates that such is not the case. This test for circulating antibody is apparently more delicate. Kendrick and her associates have applied the opsono-cytophagic test of Veitch (30) to the study of antibody production after *H. pertussis* vaccination, and during attacks of pertussis. The technic employed was relatively simple. Equal parts (0.1 cc. each) of citrated blood and a suspension of killed *H. pertussis* organisms were incubated for 30 minutes at 37° C. Smears were then made, and the number of bacteria phagocytized by twenty-five polymorphonuclear leukocytes observed. They expressed the amount of phagocytosis noted, in arbitrarily devised grades.

In a group of 117 infants and children ranging in age from 8 months to five years these workers clearly demonstrated that the degree of phagocytosis increased during and after *H. pertussis* vaccination. The maximum usually was reached two months after completion of vaccination and thereafter declined very gradually. A rather high degree of phagocytosis was still present two years later.

Kendrick and her coworkers also observed a group of 119 individuals during or after an attack of pertussis. Some of these were adults who had had pertussis many years before. During the disease progressively increasing phagocytosis was usually encountered. The maximum degree occurred in general about two months after onset. No correlation between degree of phagocytosis and severity of attack was noted. "Weak" or "moderate" reactions were observed in individuals who had had the disease 3 to 40 years previously. As controls 154 non-injected individuals with negative histories for pertussis were studied. Phagocytosis was not nearly so marked though it tended to increase with age. The white blood cells of 21 newborn infants showed practically no phagocytic power regardless of the histories or opsono-cytophagic reactions of their mothers.

Kendrick, Gibbs and Sprick (29) report uniformity of results with different Phase I strains of *H. pertussis* (that is recently isolated, "smooth" strains). It was found that the pres-

ence of a high opsono-cytophagic titer for *H. pertussis* was not associated with high titers for nine other species of organisms except *Br. bronchisepticus*. It has long been known that this organism is antigenically closely related to *H. pertussis*. The report of Kendrick, Gibbs and Sprick, however, does not conclusively show that the increased phagocytosis following pertussis vaccination is specific. The effect of injecting other vaccines on the phagocytosis of *H. pertussis* was not determined. These authors conclude that the opsono-cytophagic reactions may "offer promise of help" in the investigations of immunity in pertussis. They suggest that it may possibly act as a useful guide for immunization but "as a means for determining whether an individual has had pertussis in the past, the test is not adequate."

Using a similar technic Bradford (31) has obtained data confirmatory to the above. He notes that the heparinized blood of children with histories of pertussis is more actively phagocytic toward *H. pertussis* than that of children with negative histories.

The following studies were undertaken to see if we could confirm the findings of Kendrick, Gibbs and Sprick and to test the value of the opsono-cytophagic reaction as an index of immunity.

#### PROCEDURE

*Preparation of vaccine.* A three day growth of Phase I (smooth) strains of *H. pertussis* grown on Bordet-Gengou media was washed once in Locke's solution, and then suspended in 1:10,000 dilution merthiolate in Locke's solution. The antigen suspension was standardized to contain 20 billion *H. pertussis* organisms per cubic centimeter. This antigen was put up in 5 cc. vials and stored in the ice box.

*Technique of obtaining and preparing specimen.* Chemically clean Kahn pipettes were used. Two hundredths cc. (0.02) of 5 per cent buffered sodium citrate solution<sup>2</sup>

<sup>2</sup> The 5 per cent sodium citrate solution was buffered at pH 7.2 with Sorensen's phosphates. Evans (32) has shown that in weakly acid solution leukocytes take up H-ions and become less active. The buffered citrate solution was prepared every month and kept tightly stoppered. The final concentration of sodium citrate in the blood was 1 per cent.

It is known (33) that citrate decreases phagocytosis by binding calcium. For this reason heparin may be a better anticoagulant. The latter is advocated by Veazie and Meyer (34). Huddleson et al. (35) prefer sodium citrate for "it inhibits the action of those brucella op-



was first drawn into the pipette and immediately followed by 0.08 cc. of freely flowing blood obtained by puncture of ear lobe or heel. The specimen of citrated whole blood was then blown into small agglutination tubes (diameter 8 mm.). Within 30 minutes to 1½ hours after obtaining the specimen, 0.025 cc. of the standardized *H. pertussis* antigen was added. This blood cell antigen suspension was thoroughly mixed by very gentle rotation for one minute and then placed in the incubator at 37 degrees for 30 minutes. Without further shaking,<sup>3</sup> the blood from the bottom of the tube was drawn into capillary pipettes. A drop of this blood was placed on a thoroughly clean glass slide and smeared in the usual manner. The smears were dried in the air quickly and fixed immediately with methyl alcohol. Three to four smears of each sample were made.

*Staining of slides.* Freshly diluted Giemsa stain was found to be more satisfactory than Hastings stain or methylene blue. Giemsa stain (G. Gruber and Co.) was diluted one drop of stain per one cc. of distilled water which contained 0.001 per cent  $\text{NaH}_2\text{CO}_3$ . The slides were flooded with diluted stain for 20 minutes after which they were washed gently with distilled water and dried quickly in the air.

*Microscopic examination of slides.* The smears were examined under oil immersion. The number of organisms or absence of organisms in each of 25 polymorphonuclear neutrophils were recorded. Rarely an eosinophile containing bacteria was encountered. Frequently large mononuclears engorged with bacteria were seen. Notation of these were made; however, only the number of bacteria in the polymorphonuclear neutrophils were used in determining the opsono-cytophagic titer. Areas of the smear where clumping of either cells or bacteria occurred were avoided. Smears prepared in the manner described usually gave good preparations with an even distribution of white cells and bacteria. It was customary to examine 25 cells on each of two to three slides, thus obtaining a count of from 50 to 75 cells of each specimen of blood.

The slides were filed for examination according to number rather than name of patient. Specimens were obtained from both control and test children on the same day. All of the microscopic examinations were made by one observer who did not know the history of the patient from whom the specimens were obtained. About ¼ of the specimens were examined by an additional observer and the independent results compared.

*Clinical tests.* The technique described was used to test 150 children who were divided into the following groups.

sonins which are present in the serum of normal individuals."

<sup>3</sup> Shaking the specimen after incubation tended to increase phagocytosis, and clumping of bacteria and cells occurred frequently. Smears were more easily read if the mixture was not agitated after incubation.

Group I. Forty children were tested before and after injection of Phase I *H. pertussis* vaccine.<sup>4</sup>

Group II. Twenty-four children were tested at varying intervals after injection of Phase I *H. pertussis* vaccine. Initial or control counts were not obtained on these children.

Group III. Twenty-seven children injected with 10 cc. of *H. pertussis* "Undenatured Bacterial Antigen"<sup>5</sup> were subjected to these tests.

Group IV. Thirty-two control children were tested. Fifteen of these received three injections of 1 cc. normal saline and seventeen received no injections. Many of these children had more than one test after intervals of one to four months.

Group V. Twenty-eight children were tested during the course of pertussis.

## RESULTS

### Group I

The forty children tested before and after injection of Phase I vaccine fell into the following age groups:

4 months to 6 months .....	4 children
6 months to 12 months .....	14 children
12 months to 18 months .....	10 children
18 months to 24 months .....	2 children
2 years to 5 years .....	7 children
5 years and over .....	3 children

The initial test was done on the day of the first injection of vaccine. This total count per 25 cells ranged between 16 to 440 bacteria with a mean of 114. The per cent of cells participating in phagocytosis was between 30 and 100 per cent with a mean of 60 per cent. In all instances in which the initial total count per 25 cells was over 200, it was noted that the children fell into groups over 18 months of age. It should not be inferred from that that *all* older children give high titers since very low titers were not infrequently observed in older children.

The second test was done on the day of the third injection of vaccine (two weeks after the first injection). Thirty-nine of the 40 children were tested. The total counts of bacteria ranged between 172 to 1385 organisms per 25 cells with a mean of 637. Following injection of vaccine, successive tests showed practically 100 per cent of the cells participated in phagocytosis in all cases.

Twenty-two of the children were given a third

<sup>4</sup> Kindly supplied by Cutter Laboratories from strains isolated by the authors.

<sup>5</sup> Kindly supplied by Eli Lilly Co.

test about two months after their last dose of vaccine. The total counts of this test ranged between 116 and 1830 bacteria per 25 cells with a mean of 916 bacteria.

The fourth and last test was taken on fourteen of the children at intervals varying from three to six months after their last dose of vaccine. The total counts of this test ranged between 758 and 2985 organisms per 25 cells with a mean of 1608 organisms.

These studies were continued over a six month period using the same lot of *H. pertussis* bacterial suspension. During the last two months of the study a higher initial or control count was observed frequently. It was thought that ageing may have been a factor altering the antigen suspension and producing this effect. Three new lots of antigen were prepared simultaneously using three different strains of freshly isolated Phase I *H. pertussis*. These were checked with the first antigen used throughout the six month period. It was found that the new lots of antigen varied considerably with each other as well as with the original old antigen when tested on the same individual's blood. Obviously, subsequent tests on any of the forty children of this group using the new antigen would not be comparable. These blood studies were discontinued and the data on hand obtained from the one lot of antigen were subjected to the following statistical analysis.

To determine whether an alteration of the antigen suspension occurred with ageing, the data obtained on the 1st, 2d, 3d, and 4th test were divided into the tests done during the first four months and the tests done during the last two months. The mean, standard deviation, standard error of the means, standard error of the standard deviation and standard error of the difference of the means of the total counts of organisms per 25 cells were calculated for each of these two periods.

The difference of the means and standard error of the difference of the means between the results obtained during the first four months and those obtained during the last two months for the 1st, 2d, 3d, and 4th test show that while apparently some alteration of the antigen had occurred with age (indicated by the increase in the means of the

TABLE I

*Comparison of counts of the number of bacteria ingested by 25 cells in successive tests during the first 4 months and last 2 months' periods*

	First 4 months of use of antigen. Mean counts with standard errors	Last 2 months of use of antigen. Mean counts with standard errors	Difference between means with standard error of differences
Initial or control test (1st test)	$N^*=29$ $83 \pm 15$	$N=11$ $189 \pm 52$	$106 \pm 70$
14 days after first injection (2d test)	$N=23$ $514 \pm 43$	$N=16$ $813 \pm 77$	$298 \pm 77$
2 months after last injection (3d test)	$N=14$ $703 \pm 53$	$N=8$ $1289 \pm 212$	$587 \pm 219$
3 to 6 months after last injection (4th test)	$N=3$ $1172 \pm 139$	$N=11$ $1727 \pm 211$	$655 \pm 253$

\*  $N$  = Number of cases upon which the mean is based.

second series over the first) (Table I), these differences were not statistically significant, a fact probably dependent on the smallness of the samples.

Calculations are shown in Table I.

Therefore, because no significance could be attached to the differences in means, although consistently in favor of the older antigen, it was decided to determine the means and standard deviations of the total series for each successive testing by counts. Summary of results of these calculations is shown in Table II. The difference of means, between the first and second, second and third, and third and fourth determinations (indicated by figures in italics in Table II), are

TABLE II

*Differences in titers on successive tests of all cases during a six month period of study*

	Number of cases	Means ( $M$ ) of total number of bacteria per 25 cells with standard errors	Standard deviation of total count per 25 cells with standard errors	Differences of means — with standard errors of differences
Initial or control (1st test)	40	$M_1=114 \pm 19$	$118 \pm 13$	$M_2 - M_1$ 523 $\pm$ 44
14 days after 1st injection of vaccine (2d test)	39	$M_2=637 \pm 40$	$250 \pm 28$	$M_3 - M_2$ 279 $\pm$ 93
2 months after last injection of vaccine (3d test)	22	$M_3=916 \pm 83$	$390 \pm 59$	$M_4 - M_3$ 692 $\pm$ 185
3 to 6 months after last injection of vaccine (4th test)	14	$M_4=1608 \pm 166$	$612 \pm 116$	

found to be statistically significant. Each difference is greater than three times its standard error.

Correlations between the first and second, second and third, first and third, and first and fourth tests were calculated using the Pearson co-

efficient  $r_{xy} = \frac{\sum xy}{n\sigma_x\sigma_y}$ . Since all of the coeffi-

cients were low and not statistically significant, the calculations show that a child with an initial high titer will not necessarily give a proportional higher titer on successive tests following injection of Phase I *H. pertussis* vaccine.

### Group II

Twenty-four children who had received injections of *H. pertussis* vaccine before this study was undertaken were brought into the clinic for testing. Fifteen of the twenty-four children were under 18 months of age. Tests were repeated at intervals of several months on most of the children. While results of tests on children without initial or control counts would not be of significance by themselves, it was felt that they might prove to be of value when compared with the tests of control children in a similar age group. The results are summarized in Table III.

TABLE III  
Titer of children in Group II

	Interval between injection of vaccine and tests			Total
	0 to 2 months	3 to 6 months	6 to 9 months	
Number of children tested . . .	15	19	8	42
Average total number of bacteria per 25 cells . . . . .	617	714	942	760

There were several children in Groups I and II who persisted in showing low total counts on successive tests following vaccine injection. The significance of this is not known. These children will be followed closely in the future to determine whether there is any correlation between opsono-cytophagic titer and susceptibility to pertussis.

### Group III. Undenatured bacterial antigen

Twenty-eight children who received a total of 10 cc. of *H. pertussis* undenatured bacterial anti-

gen (Krueger) were tested. Fifteen of the twenty-eight children were under 18 months of age. Seven of these children had initial or control counts. The rest of the children had one or more tests at varying intervals. The resulting total counts of these children did not vary perceptibly with the time lapsing between the injections of undenatured bacterial antigen and the test. The results are summarized by age groupings in Table IV. The variation of total count following injection of undenatured bacterial antigen in relation to time interval after injection is shown in Figure 2 (composite scatter diagram of all groups).

TABLE IV  
Titers of children in Group III

	Under 6 months of age	Between 6 and 12 months of age	Between 12 and 18 months of age	Between 18 and 24 months of age	Over 2 years of age	Total
Number of children . . .	3	10	5	2	8	28
Number of tests done . . .	11	26	11	2	14	64
Average total number of bacteria per 25 cells . . . . .	58	53	141	92	148	93

### Control Group IV

Thirty-two children were used as controls. Seventeen of these had no injections and 15 of these received three injections of 1 cc. each of normal saline. Nineteen of these children were under 18 months of age. Tests were repeated at varying intervals. Results are summarized according to the age of the child in Table V.

TABLE V  
Titer of children in Group IV

	Age of child tested					Total
	Under 6 months of age	Between 6 and 12 months of age	Between 12 and 18 months of age	Between 18 and 24 months of age	Over 2 years of age	
Number of children tested . . . . .	13	2	4	3	10	32
Number of tests done . . .	26	3	5	9	12	55
Average total number of bacteria per 25 cells . . . . .	44	195	87	121	152	91

In Groups II, III, and IV, the number of children tested and the number of children in each group under eighteen months of age is very similar. Comparison of the total results of each group (II—average 760 bacteria per 25 cells; III—average 93 bacteria per 25 cells; IV—average 91 bacteria per 25 cells), clearly shows that the opsono-cytophagic titer for *H. pertussis* is increased following injection of vaccine. Krueger's undenatured bacterial antigen does not produce an increase in titer. Titers of children injected with the undenatured bacterial antigen are almost identical to titers of the control children.

#### Group V. Test for specificity

We wished to determine whether *H. pertussis* Phase I vaccine was the only agent which would increase the opsono-cytophagic titer for *H. pertussis*. In order to do this seven children were injected with a mixed respiratory vaccine containing *Staphylococcus aureus* and *albus*, streptococcus, *H. influenza*, *M. catarrhalis*, pneumococcus and Friedlander bacillus in a total count of one billion per cc.

Because of the tendency to produce local reactions, amounts of 0.1 to 0.3 cc. of the respiratory vaccine were injected. The ages of these children ranged between 6 months and 6 years. Results of the tests before and after injection are summarized in Table VI.

It will be noted that in five children a definite increase in titer occurred soon after injection of mixed respiratory vaccine. This will be discussed later.

TABLE VI  
Titers of children in Group V

Case	Age of child	Number of <i>H. pertussis</i> bacteria per 25 cells		
		Control test before injection	2d test 2 weeks after 1st dose vaccine	3d test 2 to 5 months after injection of vaccine
(1) R. D.....	2 years	88	382	200
(2) S. P.....	17 months	15	8	
(3) S. F.....	4 years	25	123	484
(4) E. H.....	8 months	46	1231	422
(5) R. U.....	6 years		422	484
(6) S. T.....	6 months	86	871	
(7) J. M.....	6 years	119	814	

#### Group VI. Studies during the course of pertussis

Twenty-eight children and one adult were tested during their course of pertussis. One to seven tests were done on each individual. Two children of this group had tests 3 months prior to the onset of pertussis. One of these was injected with 10 cc. undenatured bacterial antigen eight months before the onset of pertussis. The other child received injections of saline and served as a control for the undenatured bacterial antigen. One adult, mother of children with pertussis, was tested 7 days before she developed the disease. It would have been desirable to have tested all of these twenty-eight children before the onset of pertussis as well as during the course of the disease. Unfortunately, we were not able to predict the onset of pertussis in advance.

Results of the three patients mentioned are shown in Table VII.

TABLE VII  
Titers of three patients with pertussis

Patient	Age	Total bacterial count per 25 cells
V. K. Received 10 cc. of undenatured bacterial antigen 8 months prior to onset	years 1 1 4/12 1 5/12	40—3 months before onset of pertussis 327—7 days after onset of pertussis 578—35 days after onset of pertussis (5 days after cessation of cough)
M. L. K. Saline Control	2 2 4/12 2 5/12	109—3 months before onset of pertussis 203—7 days after onset of pertussis 520—35 days after onset of pertussis (7 days after cessation of cough)
Mc. C. B.	45	223—7 days before onset of pertussis 301—12 days after onset of pertussis 600—27 days after cessation of cough

These three patients had mild cases of pertussis. The results in Table VII are probably a fair sample of the change in titer during the course of the disease. The results of all of the tests done on the children during the course of pertussis are shown in Figure 1, a scatter diagram in which the opsono-cytophagic titer is plotted against days from onset and days after cessation of cough. The marked increase during the course of the disease and persisting for six weeks after cessation of cough is clearly shown in this scatter diagram. For comparison, the average of all titers obtained from children before treatment with pertussis antigen are shown in space at the left.

In order to compare more easily the titers re-

sulting from tests on children in Groups I, II, III, IV, and V, a composite of the results is shown in a scatter diagram (Figure 2). On this figure the titers are plotted according to interval lapsing between treatment and test.

Observing the total of 94 control tests (which includes initial or control titer of Groups I, II, III, and V as well as all the titers of control Group IV) the wide individual variation of titer is brought out. Eighty-seven per cent of these fall below 200 organisms per 25 cells. The 12.8 per cent of control titers above 200 bacteria per 25 cells were obtained from children over 18 months

of age. Likewise, it will be noted that 41 of the 46 tests (89 per cent) made on children injected with undenatured bacterial antigen (indicated by a bar) also fall below a titer of 200, regardless of the interval lapsing between treatment and test. *H. pertussis* undenatured bacterial antigen apparently does not increase the opsono-cytophagic titer for *H. pertussis*. Titers of this group are very similar to the controls. The high range of titer indicated by the dots, circles and "R" connecting lines clearly demonstrates the marked rise of titer following injection of Phase I *H. pertussis* vaccine and mixed respiratory vaccine.

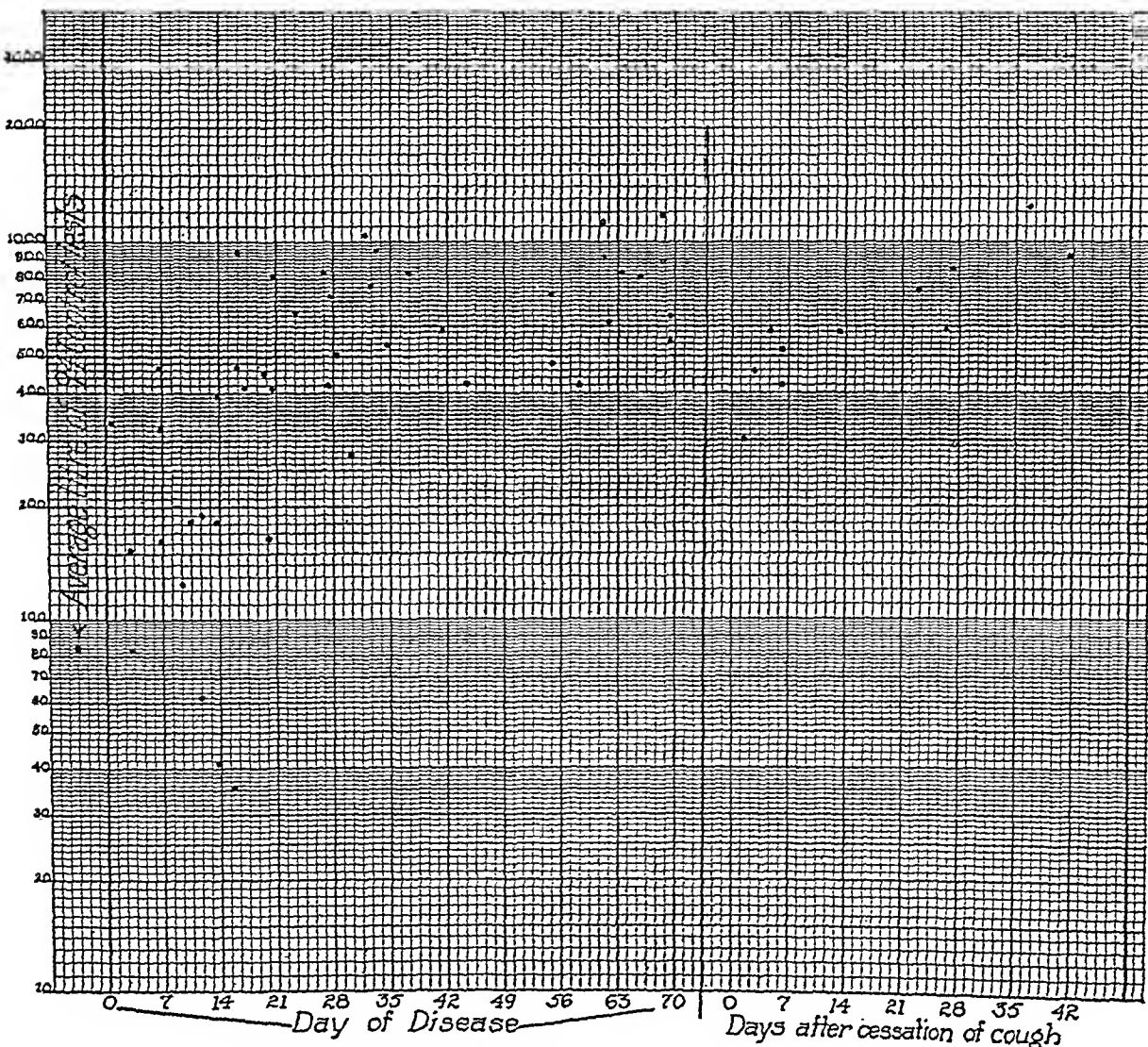


FIG. 1. ORDINATE—PHAGOCYTIC TITER (TOTAL NUMBER OF *H. pertussis* PHAGOCYTIIZED BY 25 LEUKOCYTES) PLOTTED LOGARITHMICALLY. ABSCISSA—DAYS OF THE DISEASE FROM ONSET AND DAYS AFTER CESSATION OF COUGH



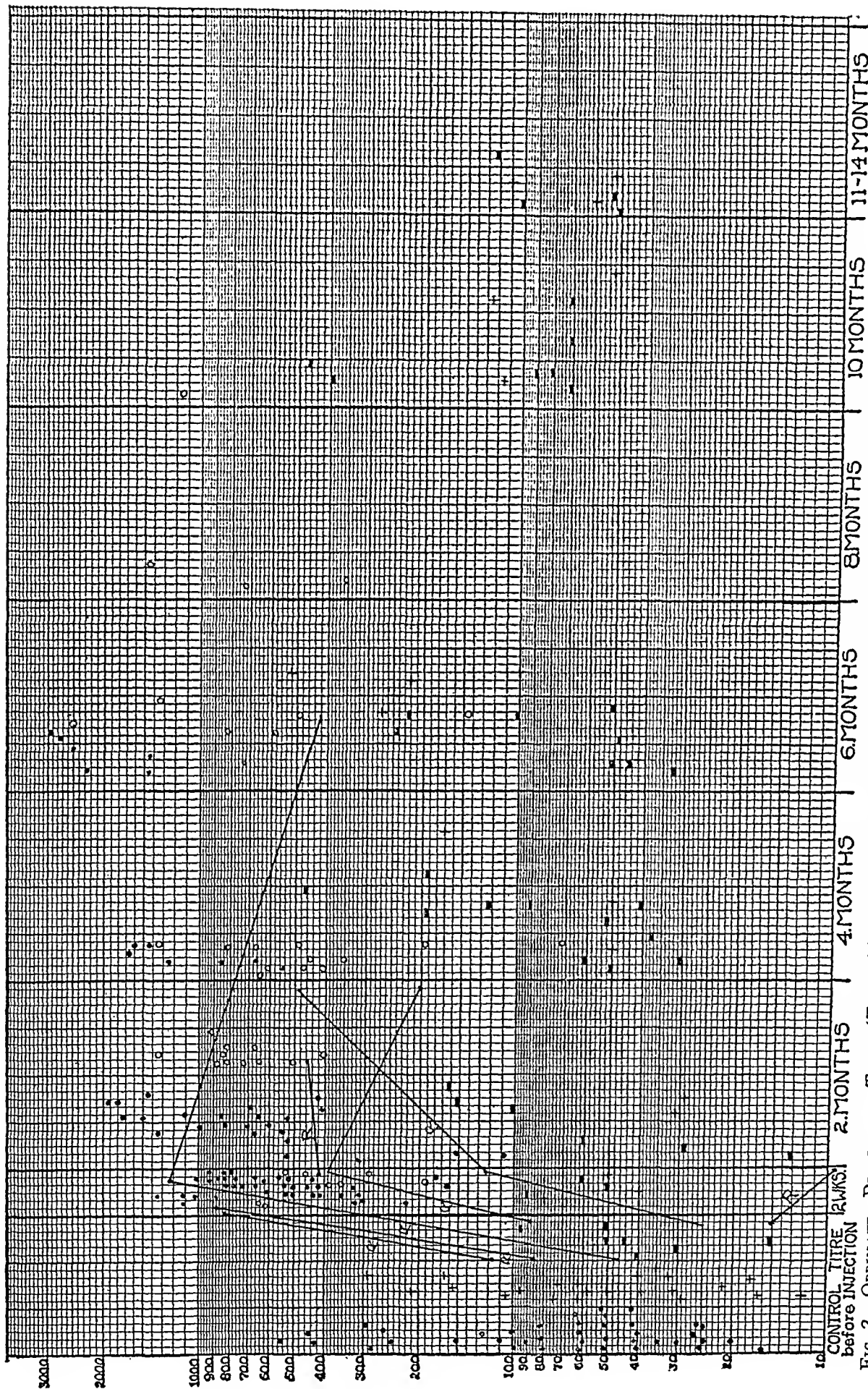


FIG. 2. ORDINATE—PHAGOCYTIC TITER (TOTAL NUMBER *H. pertussis* PHAGOCYTIIZED BY 25 CELLS), PLOTTED LOGARITHMICALLY. ABSCISSA—INTERVAL OF TIME LAPSE BETWEEN THE FIRST (PRE-TREATMENT) TEST AND SUCCESSIVE TESTS

The dots indicate titer of children in Group I (tested before and after injection of *H. pertussis* Phase I vaccine). The circles indicate titers of children in Group II (tested subsequent to injection of Phase I *H. pertussis* vaccine: no initial or control count). Bars indicate titers of children in Group III (injected with *H. pertussis* undenatured bacterial antigen). Crosses indicate titers of control children in Group IV. Since Group V (injected with mixed respiratory vaccine) was small, the titers are indicated by "R" connecting lines to show individual increases or decreases. Note that after injection the dots are found in the high range; circles show a similar distribution, whereas the crosses and bars remain in the range similar to all initial titers.

DISCUSSION <sup>a</sup>

The variables in this test may be listed and discussed as follows;

*A. Immunological variables*

1. *Specific opsonin.* The immune body for which we are testing sensitizes *H. pertussis* rendering the latter more susceptible to phagocytosis, not only by circulating polymorphonuclear leukocytes but by sessile phagocytes. If specific opsonin was the only variable involved the opsono-cytophagic test would be a direct measure of circulating immune body, which manifests itself more readily by sensitizing the bacterium for phagocytosis, than by agglutinating the bacterium, or fixing complement in the bacterium's presence.

2. *Normal opsonin.* Wells (38) noted a rapid decrease in normal opsonin in the sera of infants during the first four weeks of life. Tunncliffe (39) confirmed this observation and found that not until the end of the second year does the titer regain the average level of that for adult serum. It is obvious that this knowledge of the variation of normal opsonin with age should be considered in discussing our findings. Kendrick,

<sup>a</sup> A review of the following definitions may be of interest.

1. The *phagocytic index* (36)—the average number of bacteria phagocytosed per leukocyte (50 or 100 leukocytes are usually counted).

2. The *opsonic index* (36)

$$\frac{\text{Phagocytic index of leukocytes in the test serum}}{\text{Phagocytic index of same leukocytes in a "normal" serum}}$$

It is a test for antibody.

3. The *cytophagic index* (37)

$$\frac{\text{Phagocytic index of leukocytes to be tested in a given serum}}{\text{Phagocytic index of control leukocytes in the same serum}}$$

It is a test for the inherent phagocytosing power of leukocytes.

4. The *opsono-cytophagic index* (37)

$$\frac{\text{Phagocytic index of leukocytes to be tested in serum to be tested}}{\text{Phagocytic index of control leukocytes in control serum}}$$

Leukocytes and sera need not be added separately. Whole blood may be used. It is a test for antibody plus phagocytosing power.

Gibbs and Sprick (29) noted increased phagocytosis of *H. pertussis* as age advanced, and our findings are confirmatory. Furthermore, our observations of increased phagocytosis of *H. pertussis* after injections of mixed respiratory vaccine can be interpreted only on the basis of an increase in normal opsonin. The question then arises, is the increased phagocytosis of *H. pertussis* after *H. pertussis* vaccination due to an increase in normal opsonin with probably little real increase in resistance, or, is the observed increased phagocytosis due to formation of specific opsonin which is an indicator of immunity? This question might be answered by making determinations of the opsonizing power of inactivated serum on the washed leukocytes of infants. The use of whole citrated blood in the opsono-cytophagic test leaves the question unanswered.

3. *Variations in the inherent phagocytic activity of leukocytes.* Glynn and Cox (37) showed that the phagocytic power of leukocytes is subject to variations in the same individual. There was no evidence that the variations were specific in the uninfected. Tunncliffe (39) found that "leukocytes at birth are a little less active than those of adults. Their activity diminishes considerably during the first months of life and does not regain that of leukocytes of adults until about the third year." Here again, as with normal opsonins, one must consider a gradually rising base line under the age of three years when one measures degrees of phagocytosis. However, the increase in phagocytosis of *H. pertussis* which we observed after vaccination and during the disease is far too great to be attributed to an increasing inherent activity of leukocytes. Our control data indicate this.

Since the test as here described involves these three variables, the degree of phagocytosis in each test is the product of the variables. At the same time it seems more rational to test for the product of these three factors than for any one of them individually. This product is what Tunncliffe (39) has termed "the anti-infectious power of the blood."

*B. Technical variables*

The test is not rendered any simpler by the following technical variables and difficulties.

1. *Variations in the number of leukocytes available in the blood antigen mixture.* Abnormal or even wide variations in the total white blood counts of the patients tested will influence the degree of phagocytosis observed. Fleming (40) has pointed out that the fewer the available leukocytes in the blood sample, the greater will be the number of bacteria ingested by an arbitrary number of leukocytes.

2. *Interference by agglutination.* With the present technique of the test, allowing incubation of the blood and bacterial suspension for 30 minutes, agglutination of the organisms is sometimes observed. Clumps of agglutinated bacterial cells are not seen within the leukocytes, though they are noted sticking to the outer surface of the leukocytes. If agglutination is marked, the number of bacteria observed within 25 leukocytes will be low. Conceivably, if a longer incubation period were used, time for phagocytosis of these clumps might be available. (It seems probable that *in vivo* the macrophages are concerned with the phagocytosis of agglutinated organisms.) This interference of phagocytosis by agglutination is an important drawback to the test. In the above experiments it certainly accounts for some of the wide individual variations.

3. Kendrick et al. (29) state that all Phase I *H. pertussis* strains used in their tests gave consistent comparable results. In our experience, however, different smooth *H. pertussis* strains gave considerable variation in titer when used to test the same individual's blood. This factor is a distinct handicap in continuing these studies over long periods of time.

4. *The age of the bacterial suspension used.* As is mentioned above, we found that as the suspension aged the bacterial cells were more readily phagocytized. Kendrick, Gibbs and Sprick did not have this difficulty. It may be explained by denaturation of the bacterial protein.

5. *Variations in the time interval* between drawing the blood and adding the bacterial suspension apparently were not important factors in our experience. When the antigen was added one hour after blood was obtained, the degree of phagocytosis noted was similar to that when the antigen was added immediately. Veitch (30), however, rightly insists on a standard time interval.

After considering these inherent and technical variables it is obvious that the experimental error is great. Because of this the writers feel that little significance can be attached to a single determination. It does not seem wise to grade results as Kendrick, Gibbs and Sprick (29) did into negative, weak, moderate and strong reactions on the basis of enumeration of bacteria ingested, except in relation to a change in degree. The actual amount of phagocytosis observed is far less important than the increase observed with a given technique.

It should be mentioned that the opsono-cytophagic test has proved to be of value in the diagnosis of brucellosis. Huddleson et al. (35) have reported a high specific opsono-cytophagic index occurring in convalescents and also in individuals in contact with infected cattle and infected meat (dairy and slaughter house workers). Meyer et al. (41) found high indexes in 21 of 22 active cases.

Recently Ward and Lyons (42, 43) have emphasized the importance of phagocytosis in resistance to streptococcal infections. They make the statement that the leukocyte in the presence of the type specific opsonin is the basis of streptococcic antibacterial immunity. Lyons (44) has utilized the opsono-cytophagic test as a means of determining the presence of specific opsonin in the sera of donors to be used for transfusion. Donors are then selected for the treatment of septic streptococcic infections on the basis of the opsonin content of their sera.

Our determinations on the opsono-cytophagic power of the blood after *H. pertussis* vaccination and after an attack of pertussis confirm the work of Kendrick, Gibbs and Sprick (29). We agree that the opsono-cytophagic test may be of value in estimating the potency of immunizing agents. We also agree that it seems inadequate as a test of immunity. To their report, however, we would append the following remarks:

1. Different strains of *H. pertussis* seem to vary in the ease with which they are sensitized and phagocytized by a given blood.

2. We have obtained evidence suggesting that the increased phagocytosis of suspensions of *H. pertussis* observed during *H. pertussis* vaccination may not be entirely due to the formation of specific opsonin. The observation that injections of



a mixed respiratory vaccine increased the phagocytosis of *H. pertussis* indicates that this organism is readily sensitized by nonspecific antibody (normal opsonin). This detail suggests that immunity to pertussis is not entirely specific. At the same time this detail may not be a theoretical disadvantage to the test.

The value of the opsono-cytophagic test depends entirely on the correlation between a marked degree of phagocytosis and immunity to adequate exposure. This can only be determined by future observations on a large number of children. On this point, the test in its present state seems inadequate, since an isolated determination on a blood sample means little. The technique must be further improved so that with different strains similar results are obtained on a single blood specimen. The use of a standard strain dried at low temperature *in vacuo* (as by the lyophile process) might facilitate standardization.

The test, on the other hand, appears definitely more delicate than the agglutination or complement fixation reactions for determining the presence of circulating antibody. It certainly shows that antibody capable of sensitizing *H. pertussis* remains in the blood stream longer after vaccination and longer after the disease than was formerly supposed. The question of whether immunity is dependent on the presence of circulating antibody is left unanswered.

#### SUMMARY

The recent adaptation of the opsono-cytophagic test to the study of pertussis has been scrutinized, and the following findings of Kendrick, Gibbs and Sprick (29) have been in general confirmed.

1. After injection of infants and children with a vaccine of *H. pertussis* in Phase I there was a definite increased phagocytosis of *H. pertussis in vitro*. This high opsono-cytophagic titer was maintained for at least six to nine months.

2. During and after an attack of pertussis there was a similar rise in the phagocytic activity of the blood.

3. A low degree of phagocytosis of *H. pertussis* was found in the blood of infants under 18 months of age if they had had neither the vaccine

nor the disease. In children over 18 months of age more phagocytosis was occasionally observed even though the history was negative. It was, however, rarely as marked as after vaccination or disease.

It was also noted that:

4. After injection of *H. pertussis* undenatured bacterial antigen, no increased phagocytosis of *H. pertussis* occurred. Titers similar to those in the non-injected control children of the same age range were obtained.

5. After injection of a mixed respiratory vaccine increased phagocytosis of *H. pertussis* occurred. This stimulation of nonspecific opsonins indicates that the test is not specific for *H. pertussis* opsonins. This finding, however, does not necessarily invalidate the test, since an increase of non-specific opsonin may be commonly associated with an increase in resistance.

6. Variability of ease of sensitization and phagocytosis by the same blood was noted with different Phase I strains and with the same suspension of one strain as it aged. This together with other technical variables constitute drawbacks to the opsono-cytophagic test in its present form.

Our conclusions agree with those of Kendrick and coworkers that the test may be of value in estimating the potency of *H. pertussis* antigens when two or more determinations are made and the presence or absence of increasing phagocytosis is noted. The test at present is inadequate as a test of immunity, since immunological and technical variables obscure the value of a single determination or of multiple determinations with the same degree of phagocytosis. The ultimate value of the opsono-cytophagic test will depend on technical improvements and careful correlation with clinical immunity.

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# PLASMA CHOLESTEROL SATURATION IN PATIENTS WITH HYPERTENSION. WITH A NOTE ON PREPARATION OF GLASS FILTERS FOR MICRO-FILTRATION OF CHOLESTEROL DIGITONIDE

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Various authors during the past 25 years have attributed to hypercholesterolemia a rôle in initiating the arterial lesions of essential hypertension. The basis of this theory has been disproved by several recent papers (1, 2, 3), which have shown that plasma cholesterol, both free and esterified, is entirely normal in essential hypertension.

However, Alvarez and Neuschlosz (4) have presented a series of experiments which indicated that the serum of patients with arterial hypertension may be supersaturated with cholesterol, even

though the concentration does not exceed normal limits. Medvei (5) was unable to confirm Alvarez and Neuschlosz, and the disagreement in experimental results has remained unsettled. We have accordingly performed a series of saturation experiments on plasma from subjects with and without hypertension.

## EXPERIMENTAL

Plasma was used in preference to serum because it approximates closer to the true circulat-

TABLE I  
Plasma cholesterol values before and after saturation with free cholesterol

Patient	Hospi- tal num- ber	Age	Diagnosis	Blood pressure	Plasma cholesterol				Total lipid carbon	Free cholesterol	
					Free		Total			Degree of saturation	
					Before satu- ration	After satu- ration	Before satu- ration	After satu- ration			
CONTROL GROUP											
		years		mm. Hg	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	per cent	
R. H.....		25	Healthy	122/82	49	53	208	202			
B. M. ....		31	Healthy		62	66	220	233		94	
G. Z.....	9696	49	Pneumonia—convalescent	118/88	56	57	176	173		98	
H. Y.....	9693	40	Pneumonia—convalescent	118/80	59	59	194	196		100	
M. S.....	9723	48	Pneumonia—convalescent	110/70	50	50	196	203		100	
HYPERTENSIVE GROUP											
L. S.....	9490	53	Benign hypertension	184/112	45	41	158	162		110	
W. A.....	9399	35	Benign hypertension	117/112			269	266			
R. J.....	9452	26	Malignant hypertension	174/118	79	80	251	259		99	
A. R.....	9506	46	Malignant hypertension	260/160	56	54	175	169		104	
T. G.....	9543	37	Malignant hypertension	210/122	60	64	200	209		94	
P. M.....	9480	24	Malignant hypertension	174/108	48	45	153	149		107	
J. C.....	9839	40	Malignant hypertension	269/146	90	90	289	291	742	100	
NEPHRITIC GROUP											
L. C.....	9465	24	Chronic hemorrhagic Bright's disease	118/86	58	53	186	186		109	
F. B.....	9525	30	Chronic hemorrhagic Bright's disease	186/108	110	111	264	270		99	
		(4 hours after 100 cc. of olive oil by mouth)			134	132	306	320		101	
A. C.....	8658	22	Chronic hemorrhagic Bright's disease	240/116	42	42	133	145	396	100	
G. P.....	8740	22	Chronic hemorrhagic Bright's disease	164/100	101	100	355	354	765	101	
A. C.....	9266	30	Chronic hemorrhagic Bright's disease	124/74	152	154	432	433	1110	99	



# PLASMA CHOLESTEROL SATURATION IN PATIENTS WITH HYPERTENSION. WITH A NOTE ON PREPARATION OF GLASS FILTERS FOR MICRO-FILTRATION OF CHOLESTEROL DIGITONIDE

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Various authors during the past 25 years have attributed to hypercholesterolemia a rôle in initiating the arterial lesions of essential hypertension. The basis of this theory has been disproved by several recent papers (1, 2, 3), which have shown that plasma cholesterol, both free and esterified, is entirely normal in essential hypertension.

However, Alvarez and Neuschlosz (4) have presented a series of experiments which indicated that the serum of patients with arterial hypertension may be supersaturated with cholesterol, even

though the concentration does not exceed normal limits. Medvei (5) was unable to confirm Alvarez and Neuschlosz, and the disagreement in experimental results has remained unsettled. We have accordingly performed a series of saturation experiments on plasma from subjects with and without hypertension.

## EXPERIMENTAL

Plasma was used in preference to serum because it approximates closer to the true circulat-

TABLE I  
Plasma cholesterol values before and after saturation with free cholesterol

Patient	Hospi- tal num- ber	Age	Diagnosis	Blood pressure	Plasma cholesterol				Total lipid carbon	Free cholesterol	
					Free		Total			Degree of saturation	
					Before satu- ration	After satu- ration	Before satu- ration	After satu- ration			
CONTROL GROUP											
		years		mm. Hg	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent		per cent
R. H. ....		25	Healthy	122/82	49	53	208	202			
B. M. ....		31	Healthy		62	66	220	233			94
G. Z. ....	9696	49	Pneumonia—convalescent	118/88	56	57	176	173			98
H. Y. ....	9693	40	Pneumonia—convalescent	118/80	59	59	194	196			100
M. S. ....	9723	48	Pneumonia—convalescent	110/70	50	50	196	203			100
HYPERTENSIVE GROUP											
L. S. ....	9490	53	Benign hypertension	184/112	45	41	158	162			110
W. A. ....	9399	35	Benign hypertension	117/112			269	266			
R. J. ....	9452	26	Malignant hypertension	174/118	79	80	251	259			99
A. R. ....	9506	46	Malignant hypertension	260/160	56	54	175	169			104
T. G. ....	9543	37	Malignant hypertension	210/122	60	64	200	209			94
P. M. ....	9480	24	Malignant hypertension	174/108	48	45	153	149			107
J. C. ....	9839	40	Malignant hypertension	269/146	90	90	289	291	742		100
NEPHRITIC GROUP											
L. C. ....	9465	24	Chronic hemorrhagic Bright's disease	118/86	58	53	186	186			109
F. B. ....	9525	30	Chronic hemorrhagic Bright's disease	186/108	110	111	264	270			99
		(4 hours after	100 cc. of olive oil by mouth)		134	132	306	320			101
A. C. ....	8658	22	Chronic hemorrhagic Bright's disease	240/116	42	42	133	145	396		100
G. P. ....	8740	22	Chronic hemorrhagic Bright's disease	164/100	101	100	355	354	765		101
A. C. ....	9266	30	Chronic hemorrhagic Bright's disease	124/74	152	154	432	433	1110		99

ing fluid. Heparin was chosen as anticoagulant in order to minimize changes in cell-plasma equilibria which might conceivably affect the state of plasma cholesterol; also to avoid the saponification of cholesterol esters by oxalate or citrate, pointed out by Shope (6). The experiments were carried out at 37° C. in a further effort to approximate *in vivo* conditions.

To obtain plasma for each saturation experiment, 20 cc. of blood obtained by venipuncture were run into a 50 cc. pyrex flask containing about 10 mgm. of heparin. The blood was whirled in the flask to assure complete mixing of the heparin, poured into a heavy centrifuge tube, and centrifuged at 2500 r.p.m. for 45 minutes. After pipetting off the supernatant plasma, it was divided into two equal portions of about 5 cc. each in 30 cc. pyrex flasks. About 50 mgm. of cholesterol were added to the contents of one flask and thoroughly suspended by gentle shaking. Both flasks were then closed with rubber stoppers and placed in an incubator at 37° C. for 6 hours.<sup>1</sup> At the end of this period, the sample of plasma without added cholesterol was filtered with gentle suction through a 4 cm. Buchner funnel fitted with a close-fibered filter paper. The sample with added cholesterol was then filtered through the same paper, with care to discard the first 1 to 2 cc. of filtrate. Both filtrations were carried out in the warm room at 37° C. Free and total cholesterol determinations were made in duplicate on each sample by the method of Kirk, Page, and Van Slyke (7), with minor modifications. In a few cases, total lipid carbon was determined by the method of the same authors. The results are shown in Table I.

#### DISCUSSION

It is apparent from Table I that no pronounced changes in cholesterol content of the plasma samples studied were brought about by saturation with added cholesterol. Both free and total cholesterol remained essentially the same. Such variations as did occur are not consistent in di-

<sup>1</sup> Incubation for longer periods has no effect on the results obtained and, unless sterile precautions are taken throughout, is certain to result in contamination by bacterial growth. Mechanical agitation during the saturation period had no effect on the cholesterol content of plasma samples.

rection and therefore are probably without real significance.

#### SUMMARY

No evidence of a relationship between blood pressure and plasma cholesterol saturation was found in a series of cases which included individuals with malignant hypertension, benign hypertension, and chronic hemorrhagic nephritis. The plasma was approximately saturated with regard to free cholesterol in all cases.

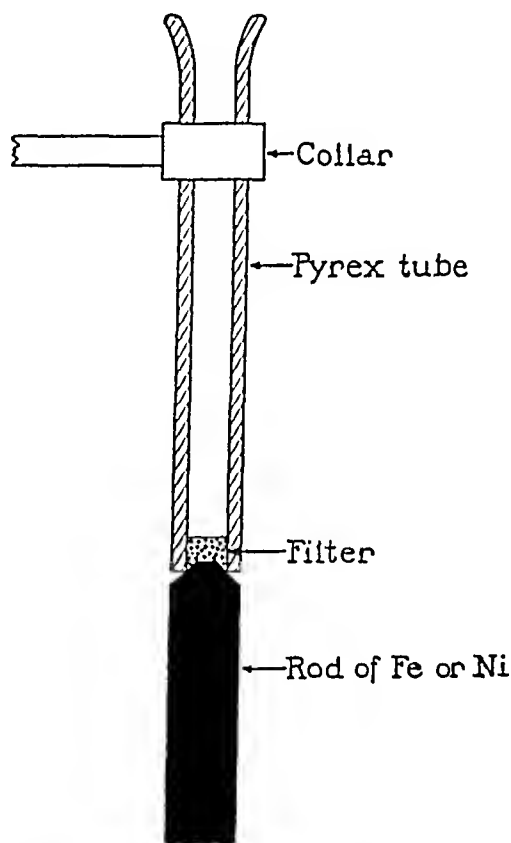


FIG. 1. METHOD FOR SINTERING GLASS FILTERS.

#### NOTE ON PREPARATION OF SINTERED GLASS FILTERS FOR MICRO-FILTRATION OF CHOLESTEROL DIGITONIDE

Kirk, Page and Van Slyke (7) used filter sticks with detachable tips for filtration and washing of the cholesterol digitonide precipitate. The filtering disk was of porous alundum. As the original supply of alundum in the laboratory was exhausted, it proved difficult to obtain more which would resist the chromic acid combustion filter for many analyses. We have accordingly changed the porous disk from alundum to sintered pyrex glass. The filter sticks can be prepared with the sintered glass disks as follows.

Bits of broken pyrex laboratory ware are ground to a powder in a large mortar. This powder is shaken on a 100-mesh screen and the screenings reserved, while the



tailings are reground and rescreened until a sufficient stock of material is obtained. The screened powder is agitated with distilled water in a small beaker and the supernatant water carrying the finest particles is decanted after a 10-second period, the process being repeated three or four times. The residual powder is then washed on to a filter paper and washed once with a saturated solution of sodium borate (borax). The paper and its contents are then dried over a steam bath. After breaking up the resultant cake into a powder again, it is ready for use.

The detachable lower end of a Kirk-Page-Van Slyke filter stick of pyrex glass is placed upright on a short iron or nickel rod as shown in Figure 1. Tubing of about 6 mm. internal diameter and 2 mm. wall thickness in lengths of about 8 cm. is used. The end of the metal rod is beveled at an angle of 45°. The bevel results in the formation of a shoulder on the finished disk, a feature of some importance.

An amount of the powdered pyrex glass prepared as above is run into the upper end of the tube, so that the upper level is somewhat above that indicated in the diagram. The matrix is then packed and smoothed by gentle tamping with a blunt glass rod. After tamping the matrix, it should have a thickness of at least 3 mm.

An air-gas blast is adjusted to deliver maximum heat intensity and brought to bear on the lower end of the tube and upper end of the metal rod, while slowly rotating about them. The upper end of the rod should reach a white heat, to insure a uniform face on the filter. The matrix will sinter at a bright red heat. The exact conditions of heating can be determined with a few trials.

If the matrix is insufficiently heated the particles in the center will not adhere to each other, while excessive heating will fuse the matrix into a solid mass. With a little practice, both of these exigencies may be avoided. The method is rapid, as the three operations of sintering a filter disk, fusing it into tubing and fire-polishing the end of the tubing are performed at one time. It is not adaptable for making filters larger than 1 cm. in diameter.

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# STUDIES ON THE MECHANISM OF PROTEINURIA

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In the chronic active stage of hemorrhagic Bright's disease there are often comparatively long intervals when the amount of functioning kidney tissue decreases very slowly and when there is no marked variation in glomerular permeability. With the patient on a standard regime the urea clearance remains constant, proteinuria fairly so, and blood samples taken under basal conditions with precautions against stasis and effects of posture contain nearly constant percentages of plasma proteins (18). Under such circumstances, some of the factors governing proteinuria may be studied.

The amount of protein in the urine, as well as its level in the circulating plasma, seems to depend largely upon the nature and severity of injury to the glomerular capillaries (14). Proteinuria may also be influenced in a moderate degree by the level of protein in the diet (4, 11, 18). In addition to these factors there are apparently others which induce temporary fluctuations in proteinuria. The observations reported here were made in an effort to clarify the problem further.

Our results support the concept that the quantity of protein lost in the urine is related to the amount of glomerular filtrate formed and to the rate of production of serum proteins.

## METHODS

*Subjects.* The investigations were conducted on four patients with chronic active hemorrhagic Bright's disease. Three of these had served as subjects for previous studies and their medical histories have been summarized elsewhere (18, 19).

The fourth patient (J. H.) was a married woman of 25 years of age. She had scarlet fever at the age of fourteen. Protein was first discovered in the urine during pregnancy at the age of eighteen. During her twenty-first year she contracted and received treatment for gonorrhea. Clinical cure of the venereal infection resulted. During her twenty-second and twenty-third years she was ill on several occasions with acute sinusitis. Two months prior to admission her face became swollen, and she was told by a physician that she had Bright's disease.

Her treatment at this time consisted of frequent cathartic doses of epsom salts and a low protein diet. A week before admission edema developed in the lower extremities and gradually extended upward to involve the abdominal wall. Physical examination revealed pallor of the skin and mucous membranes; soft pitting edema of the face, trunk and lower extremities. The systolic blood pressure was 160 mm. of mercury; the diastolic 100 mm. The heart was normal. A careful search for foci of infection was made, but except for scarred tonsils none were found. The blood, at the time of admission contained 3,200,000 erythrocytes and 8,100 leukocytes per cubic millimeter; hemoglobin 9.4 grams; nonprotein nitrogen 32 mgm., and serum proteins 3.3 grams per 100 ml. The albumin:globulin ratio of the serum proteins was 1.3. The concentration of chlorides in the serum was .112 meq. per liter; CO<sub>2</sub> combining power of the plasma was 59 volumes per cent. The urine contained much protein, many casts, red cells and epithelial cells. It was sterile on culture. An Addis sediment count revealed the presence of 13 million erythrocytes, 4 million epithelial and white blood cells, and 66 thousand casts in a twelve hour specimen. Urea clearance was approximately 40 per cent of the normal average.

The patient was given a diet liberal in calories and proteins. She lost her edema and in three weeks the erythrocytes of the blood had increased to 4,000,000 per cubic millimeter, the hemoglobin to 12.5 grams and the serum proteins to 4 grams per 100 ml. There was no further change in these values throughout the balance of her stay in the hospital. Subsequent examinations of the urine showed no marked change from those noted at the time of admission.

None of the patients on whom this report is based had clinical edema while they were subjects of investigation.

*Diets.* The diets were prepared, sampled and analyzed in a manner previously described (18). During the experiments recorded in Tables I, III and IV the patients were allowed three different menus, which varied slightly but had the same caloric and protein content and were given on consecutive days. The patients thus ate the same food every third day. During the experiments recorded in Tables II, V and VI the patients ate exactly the same food each day. No salt was added to the diets during their preparation, and fluid intake was kept constant in each experiment.

*Protein supplements* were given in powdered form. Liver protein was prepared as previously described (18). Egg white was boiled, then minced, dehydrated with alcohol, dried in a current of warm air and reduced to

powder in a ball mill. The kidney protein was prepared from fresh raw beef kidneys, which were washed in running water, then minced, dehydrated, dried and powdered as in the case of egg protein.

*Analytical methods.* All nitrogen determinations were done in triplicate. The nitrogen content of the diets, the stools and the daily urine was determined by macro-Kjeldahl. The protein in the urine of Case P. V. in Table I was determined by the method of Kingsbury, Clark, Williams and Post (16). The protein standards used, ranging from 5 to 100 mgm. per 100 ml., were checked frequently with solutions of known protein content. In all other experiments the protein of the urine was determined by the micro-Kjeldahl method described by Peters and Van Slyke (26). Serum proteins were determined by the method of Howe (26). Blood for these determinations was drawn without stasis in the morning about fourteen hours after the previous meal and while the patients were still recumbent. Plasma volumes were determined by the dye method of Keith, Rowntree and Geraghty (17) as modified by Hooper, Smith, Belt and Whipple (15). The patients were weighed at the same time each morning after emptying the bladder and prior to the ingestion of food. The stools were separated by means of carmine given at the beginning of each metabolic period. The urea clearance tests were done according to the method of Möller, McIntosh and Van Slyke (24). With the exception of those in Table V, the tests were done in duplicate under basal conditions in the morning. A uniform amount of water was given during each series of tests. The clearances in Table V were calculated from the twelve hour urea excretion according to the method of Landis and coworkers (21).

#### *The effect of the protein content of the diet on proteinuria and urea clearance*

Variations in proteinuria and urea clearance with the level of protein in the diet are recorded in Table I. In each case there were simultaneous changes in proteinuria and urea clearance. There was no constant relationship between the proteinuria and the volume of the urine.

The clearances of urea, creatinine, xylose and sucrose have all been found to vary with the level of the protein in the diet, and there is good evidence for the belief that this results from changes in the rates of glomerular filtration (32). Van Slyke, Rhoads, Hiller and Alving (33) found that increase or decrease in urea clearance was accompanied by proportional changes in renal blood flow. It, therefore, seems justifiable to interpret concomitant increase or decrease in proteinuria and urea clearance as the result of changes in renal blood flow and glomerular filtra-

TABLE I

*Changes in proteinuria and urea clearance associated with changes in level of protein in the diet*

Case	Day	Diet		Serum proteins	Urine			Standard urea clearance†
		Cal-ories	Pro-tein		Vol-ume	Non-pro-tein nitrogen	Pro-tein	
		<i>per diem</i>	<i>grams per diem</i>	<i>per cent</i>	<i>ml. per diem</i>	<i>grams per diem</i>	<i>grams per diem</i>	<i>ml. per minute</i>
P. V. March to May 1931	1-6	3400	75	7.0	1174	7.87	0.070	23
	7-12	3400	75	7.0	1312	8.30	0.090	26
	13-18	3400	150	7.3	1450	15.30	0.150	39
	19-24	3400	150	7.0	1785	17.60	0.120	42
	25-30	3400	150*		1985	17.69	0.340	
	31-36	3400	150*		1691	18.58	0.410	45
	37-42	3400	150		1809	17.50	0.410	47
	43-48	3400	10	7.0	1807	6.55	0.150	26
	49-54	3400	150		1397	15.9	0.170	45
	55-60	3400	150	7.3	964	17.4	0.270	47
L. R. October to January 1933-34	1-9	3000	70	4.1	2420	8.2	9.7	25
	10-24	3000	70	4.1	2235	7.5	11.6	20
	25-36	3000	180		2260	18.6	15.0	35
	37-81	3000	180	4.0	2220	22.3	13.0	32
	102-114	3000	70	4.0	1920	9.0	11.2	22
R. P. December 1934	1-10	3200	60	2.95				
	11-13	3200	60	2.9	1230	5.2	13.5	21
	14-22	3400	110	3.0	1220	8.4	19.0	33
	23-31	3600	160	3.0	1560	13.0	23.0	40

\* (Case P. V.) From the 25th through the 36th day, 60 grams of protein daily were furnished by liver, in substitution for beef and fish muscle given the balance of the time on the same level of protein.

† Each urea clearance value represents the mean of two or more determinations.

tion. An additional factor must be taken into consideration in Case P. V. From the twenty-fifth to the thirty-sixth days inclusive 60 grams of liver protein replaced an equal amount of beef, veal and fish protein in his diet. The substitution of liver protein led to a further rise in the amount of protein in the urine without a corresponding increase in urea clearance. Data from several sources suggest that this is due to the superiority of liver as a source of protein food from which the body can fabricate plasma protein (18, 29).

Table II shows the extent of variation in the proteinuria of Case L. R. during two days at different levels of protein intake. In each case he had received the same diet and supplement for five previous days. The patient was kept recumbent in bed during these two days to avoid postural effects. Additional experiments of this nature yielded similar results.

Proteinuria was greater while the patient was taking the higher protein diet. At both levels of intake proteinuria increased during the day. Obviously, these variations may have been due to the

TABLE II  
Variation in proteinuria during 24 hour periods in Case L. R.

Time	Hour of meals	Basal diet 70 grams protein Egg white 50 grams protein Total 120 grams protein		Basal diet 70 grams protein Egg white 23 grams protein Total 93 grams protein	
		Urine volume <i>ml. per hour</i>	Urine protein <i>grams per hour</i>	Urine volume <i>ml. per hour</i>	Urine protein <i>grams per hour</i>
<i>a.m.</i>					
6:30-8:30.....	8:40	124	0.42	100	0.38
8:30-10:30.....		58	0.50	115	0.43
10:30-12:30.....		60	0.57	168	0.49
<i>p.m.</i>					
12:30-2:30.....	12:30	83	0.56	178	0.58
2:30-4:30.....		64	0.75	239	0.49
4:30-6:30.....	6:00	67	0.92	104	0.57
6:30-8:30.....		126	0.81	115	0.55
8:30-10:30.....		74	0.78	57	0.42
<i>p.m. a.m.</i>					
10:30-6:30.....		68	0.44	57	0.36
24 hour total.....			14.13		10.68

influence of the protein eaten at meal times upon the rate of the glomerular filtration or to an increased rate of manufacture of plasma protein after meals or to a combination of these. Similar diurnal variations in the urea clearance have been found by MacKay (23), and postprandial elevations of urea and xylose clearances have been demonstrated (27, 32).

### The effect of diuretics

*Theobromine.* It is generally conceded that the xanthine diuretics increase the rate of glomerular filtration. Addis and Drury (1) and Polland (28) found that the urea excretory ratio (urea clearance) was increased by the xanthine diuretics. Schmitz has reviewed the extensive literature on the subject and added further evidence for the validity of the concept (31). Page (25) found the variation in urea clearance produced by a single dose of one of the xanthine diuretics not greater than the usual variable conditions existing in patients. This does not, however, invalidate the results obtained by previous investigators.

Data obtained when Cases R. P. and J. H. were given theobromine are recorded in Table III. The total daily dose of the diuretic indicated in the table was given in four portions at intervals of six hours.

Proteinuria and urea clearances were definitely increased above the basal level when theobromine was given. This effect continued for a day or two

after the medication was stopped. Then both proteinuria and clearance decreased to about the previous level. Proteinuria during control periods after theobromine administration was slightly less than in preceding controls, probably because of additional depletion of body protein

TABLE III  
Effect of theobromine on proteinuria and urea clearance.

Case	Day	Diet	Theobromine sodium salicylate	Urine			Standard urea clearance	Serum proteins
				Volume	Non-protein nitrogen	Protein		
		<i>per diem</i>	<i>grams per diem</i>	<i>ml. per diem</i>	<i>grams per diem</i>	<i>grams per diem</i>	<i>ml. per diem</i>	<i>per cent</i>
R. P.*	1-13	Calories 3200 Protein 60 grams	0	1000	4.5	14.7	20±3	3.2
	14		5	1490	5.9	19.6		
	15		5	900	5.4	17.1	35±2	3.0
	16		0	1200	6.2	18.9		
	17-20		0	1330	5.3	14.0	23±2	3.1
J. H.†	1-17	Calories 2500 Protein 60 grams	0	2200	7.3	4.7		4.0
	18		0	1820	7.1	4.9	23±1	
	19		4	2510	9.1	6.3		
	20		4	2120	7.2	6.5	35±4	
	21		4	1930	7.0	6.3	36±3	
	22		0	2360	8.2	6.8		
	22-24		0	1740	6.9	5.7		
	25-28		0	Lost				
	29-31		0	1620	6.7	4.4	22±2	4.1

\* R. P. From first to 13th day volume of urine varied between 640 and 1440 ml. per day. From first to 13th day protein in urine varied between 14 and 15.1 grams per day. From 17th to 20th day volume of urine varied between 990 and 1680 ml. per day. From 17th to 20th day protein in urine varied between 13.5 and 14.6 grams per day.

† J. H. From first to 17th day volume of urine varied between 1760 and 2620 ml. per day. From first to 17th day protein in urine varied between 4.2 and 5 grams per day.

during the experiment. The results suggest that theobromine caused increase in glomerular filtration.

Berglund and Sundh (5) determined the creatinine clearance and proteinuria before and after administration of euphyllin or caffeine. In most instances the results agreed with our findings. Since Berglund's observations were conducted only during an interval of three hours, the time of maximal effect of the drug may not have been included in all experiments.

Urea has been used as a diuretic for many years, and the mode of its action has been the subject of much investigation and controversy. Gottlieb and Magnus (12) and Fletcher, Henderson and Loewi (10) found evidence of increased renal blood flow after its administration. Henderson and Loewi (13) also obtained evidence of dilution of the blood. Lamy and Mayer (20) found that diuresis occurred without evidence of either of these phenomena. Cushny (8) came to the conclusion that the increase in the amount of urea in the tubules prevented reabsorption of water and that at times this effect was reinforced by increased renal blood flow and increased glomerular filtration resulting from dilution of the blood. These conclusions have been supported by the results different investigators have obtained while working with the urea clearance test. Addis and Watanabe (3) found that the administration of urea often increased the urea clearance above that obtained at normal blood urea levels. Van Slyke, Rhoads, Hiller and Alving (33) studying dogs with explanted kidneys found that the urea clearance was sometimes increased by the administration of urea, more often not. However, the changes in clearance were always found to parallel changes in the renal blood flow. There are several reports in the literature (4, 7) showing that administration of urea results in increased proteinuria, but the mechanism of its action was not ascertained. It seems likely that certain factors not yet understood, perhaps the amount of water and electrolytes available for mobilization determine dilution of the blood and expansion of its volume and thus lead to increased glomerular filtration.

Urea was administered to Cases R. P. and J. H. for several successive days while they were on a

TABLE IV

*Effect of urea administration on proteinuria and urea clearance*

Case	Day	Diet	Urea	Urine			Stand- ard urea clear- ance	Serum pro- teins
				Vol- ume	Non- protein nitro- gen	Pro- tein		
		<i>per diem</i>	<i>grams per diem</i>	<i>ml. per diem</i>	<i>grams per diem</i>	<i>grams per diem</i>	<i>ml. per minute</i>	<i>per cent</i>
R. P.	1-4		0	1330	5.3	13.8		3.0
	5		60	1830	13.4	14.8		
	6		60	2140	23.1	17.2		
	7		60	2680	27.9	19.6		
	8		0	1550	15.9	16.8		
	9-10	Calories 3200	0	1230	8.5	16.3		
	11-15	Protein 60 grams	0	1350	4.5	14.3	20±3	
	16		40	1530	12.0	15.5		3.1
	17		40	1640	14.4	15.5		
	18		40	1940	20.7	17.6	31±2	
	19		0	Lost				
	20		0	1310	12.6	14.0		
	21-23		0	1030	7.8	13.4	23±0	3.1
							Maxi- urea clear- ance	
J. H.	1-10		0	1795	6.5	4.9	32±1	4.0
	11		40	1890	14.9	6.9		
	12		40	2690	21.9	7.1	61±6	
	13		40	2530	21.2	7.2	56±2	
	14		40	2740	34.0	7.9	54±1	
	15	Calories 2500	0	2790	32.5	7.4		
	16	Protein 60 grams	0	1670	20.2	7.2	53±2	
	17-19		0	1380	10.4	7.4		3.9
	20-24		0	Menses				
	25		0	1170	7.7	4.4	35±2	4.0

constant dietary and fluid intake. It was necessary to give 100 ml. of extra water with each 10 grams of urea. The data are recorded in Table IV. In each instance the urine volume rose above the control level when the larger amounts of urea were excreted. At times the increase in urine was greater than the extra water administered; therefore, fluid must have been withdrawn from the body. The smaller urine volumes during after periods are indications of readjustment.

Proteinuria and urea clearance increased. Both of these effects we believe to have been manifestations of increased glomerular filtration. The increase in proteinuria in Case J. H. attained a maximum of 61 per cent above the control level and remained high until the excess of urea had been eliminated, a matter of some three or four days after its administration had been stopped. As will appear later, there is a rather striking parallelism between increase in proteinuria after urea and after the ingestion of a large supplementary feeding of protein.

TABLE V

*Protein metabolism before and after intravenous plasma protein in Case R. P.*

Period	Day of period	Diet and remarks	Protein intake	Urine non-protein nitrogen	Stool nitrogen	Urine protein	Balances	Blood			Plasma volume	Urine volume	Standard urea clearance	Body weight
								Non-protein nitrogen	Serum albumin	Serum globulin				
5 days each			grams of nitrogen per diem	grams per diem	grams per diem	grams of nitrogen per diem	grams of nitrogen per diem	mgm. per cent	per cent	per cent	ml.	ml. per diem—Average	ml. per minute	kgm.
1-3	Average	Control diet 2500 calories 67 grams protein	10.67	7.88	1.34	1.47	+0.18	35	2.46	1.83		1415	28	61.84
4-7	Average		10.67	6.92	1.34	1.40	+1.04	35	2.35	1.63	3060	1632	28	61.07
8-11	1		10.67 10.67	6.72 6.67	1.34 1.34	1.47 1.42	+1.14 +1.24	35	2.56*	1.57	2820	1640 1658	25	61.64 61.53
12	2	Control diet plus Plasma protein 41 grams Plasma nonprotein nitrogen 0.16 grams	10.67 6.72					36	2.94** 3.03†	1.51 1.65				
		Intravenously	17.39	7.28	1.34	2.09	+7.78	33	3.08‡	1.55	3850	2270	38	61.31
			10.67 10.67 10.67 10.67	6.85 6.31 6.07 6.24	1.34 1.34 1.34 1.34	2.52 2.46 2.17 2.04	+0.31 +0.56 +1.09 +1.05	32	2.62	1.80	3050	1720 1575 1515 1560	27.5 27	61.38 61.42 61.48 61.68
13	3	Control diet	10.67	6.71	1.34	1.89	+0.73	32	2.77	1.60		1693	27	61.76
14	Average		10.67	6.49	1.34	1.74	+1.10					1600		62.05
15	Average		10.67	6.82	1.34	1.70	+0.80	35	2.52	1.68		1650		62.29

\* Before transfusion.

\*\* 10 minutes after transfusion.

† 6 hours after transfusion.

‡ 21 hours after transfusion.

*The effect of increasing the volume of the plasma and the level of plasma protein by transfusion*

The immediate effect of transfusion of plasma protein was found to be an increase in the concentration of protein in the plasma and an increase in plasma volume. The data from such an experiment are recorded in Table V. During a preliminary period of thirty-four days Case R. P. was kept on a constant diet, the protein and caloric content of which was fixed at a level found to be sufficient to permit the daily deposition of a small amount of protein. The patient's activity was limited to walking about his room. When catabolism, proteinuria and protein deposition were all fairly constant (Period 12) he received a transfusion of 783.5 grams of citrated plasma from compatible donors. The actual volume of the transfusion was 771 ml. On analysis the transfusion mixture was found to contain 6.56 grams of protein nitrogen and 0.16 gram of nonprotein nitrogen. Ninety-five ml. of physiological sodium chloride solution were used to wash the plasma from the gravity apparatus into the vein.

Previous to transfusion the concentration of serum proteins was nearly constant at 4.0 to 4.1

grams per cent. The maximum rise after transfusion was noted at the end of 6 hours, when a concentration of 4.68 grams per cent was recorded. Measurement of the plasma volume 21 hours afterward showed an increase about equivalent to the quantity of fluid injected.

Proteinuria increased and remained at levels higher than control for fourteen days. At the end of this time, the concentration of serum protein had fallen to the pretransfusion level, and the sum of the daily increments in proteinuria had now slightly exceeded the total amount of transfused protein. The urea clearance rose during the period of increased plasma volume and, it seems logical to infer increased glomerular filtration until the second day after transfusion, when it was found that urea clearance and plasma volume were essentially as in control periods. Increases in proteinuria thereafter were presumably associated with the slight increase in the concentration of plasma protein.

*Lag in proteinuria*

It has been observed that a change from a lower to a higher level of protein in the diet is some-

*Protein metabolism and proteinuria as affected by large supplementary feedings of protein given during a single day*

[illegible]



times followed by a prompt increase in proteinuria; at other times there is a delay before the increase in urinary protein appears. Whipple and collaborators have noted a similar delay in formation of plasma proteins in the dog, and attributed it to filling of reserve depots previously depleted of protein (29). Falta found considerable lag in excretion of nitrogen following superposition of certain proteins on a control diet (9). Lag was attributed to deposition of protein in the tissues and subsequent catabolism of it.

Table VI shows the effect of adding a large quantity of protein to a standard diet during a single day. Supplementary feedings of egg white, kidney and liver protein were given. In each case the amount of protein fed contained 16 grams of nitrogen. Catabolism of protein, as represented by the nonprotein nitrogen of the urine, was greatest on the day of ingestion of extra protein or on the following day and gradually declined to the control level three or four days later. Any diuretic effect<sup>1</sup> of the protein supplement should have been operative during this interval. As the table indicates, proteinuria was usually at a maximum a day or two following the day on which the maximum excretion of nonprotein nitrogen occurred. After ingestion of liver protein both patients continued to excrete a considerable amount of excess protein in the urine, even when the urinary nonprotein nitrogen had returned to basal values. No evidence of increase in the concentration of serum protein was found, an observation in agreement with previous determinations of plasma proteins during such periods of increased proteinuria (18). The delay in attainment of maximum proteinuria was similar to that observed when urea was administered. In the absence of sufficiently complete data an hypothesis may be tentatively offered to explain the delayed rise in urinary protein on feeding liver protein and the persistence of this rise after evidence of increased catabolism of protein had disappeared. On feeding urea, it was noted that the full effect on glomerular filtration in terms of proteinuria was not achieved until the second or third day of the experiment. This is probably because time is required for the expansion of plasma volume pre-

ceding increased renal blood flow. Furthermore, it has been clearly shown that in dogs undergoing plasmapheresis an increase of protein in the ration increases the formation of plasma protein and that this effect persists for a varying interval of time after the protein supplement has been discontinued (29). In the present instance both of the aforementioned factors may have been operative. The long delay in reverting to basal conditions is probably due to increased production of plasma protein. When the tissues receive the components of plasma protein, an interval of two or three days may elapse before the synthesis of new protein is completed. Thereafter, it may be picked up slowly by the circulating plasma and escape in the urine. This is equivalent to stating that protein is first deposited in a depot and subsequently given up to the blood.

#### DISCUSSION

Despite gaps in our knowledge, currently recognized factors give a fairly clear picture of the mechanism of proteinuria. The rôle played by increase in glomerular permeability with resultant fall in the level of plasma proteins, the latter effect in turn leading to a heightened stimulus for the formation of these proteins, has already been discussed (18). The response to the stimulus leading to production of plasma proteins is partly dependent upon dietary sources and partly upon available stores of protein in the tissues. The amount of protein which can ultimately be contributed to the plasma by the tissues is evidently very large, but the readiness with which the tissues give up protein depends upon the presence of a labile reserve of protein in them. This seems to be but a relatively small fraction of the total protein of an organ or tissue. It may be rapidly depleted when plasma protein is needed and conversely quickly restored under optimal nutritional conditions. This fraction has been thought of in the past as a separate entity or depot, but the work of Luck (22) probably establishes its existence as an integral part of the cellular structure of the body, indistinguishable from other tissue proteins. The existence in the liver of such labile protein has been demonstrated by Addis, Poo and Lew (2). Replacement of this labile protein or the fact that plasma proteins must pass through the labile protein stage may be responsible for the lag

<sup>1</sup> The term "diuretic effect" as used above is synonymous with increased glomerular filtration.

in their appearance in the urine under favorable nutritional conditions. Data in Table VI suggest that protein may at times be temporarily retained in the labile fraction during the process of being converted into circulating plasma protein.

The balance of the protein of the body seems to be more firmly bound. Even large additions to tissue and organ proteins do not seem to influence the concentration of plasma proteins nor proteinuria (18). Yet a small addition to labile stores is reflected in an increased proteinuria, presumably through the medium of increase in the rate of formation of plasma proteins. When the store is exhausted, proteinuria returns to its previous level.

The rate of glomerular filtration has a definite influence on the magnitude of proteinuria. Variations in the rate of filtration may be brought about by changes in protein intake or by other changes in protein metabolism which Pitts (27) has found to be intimately associated with changes in renal activity. In the experiments in this paper, variations in the rate of glomerular filtration were produced by diuretic substances, by increasing the volume of blood and by changing the protein content of the diet. It is only by the use of diuretics that one may clearly separate the effect of changes in the rate of glomerular filtration from other factors which tend to change proteinuria. We have used the urea clearance as evidence of change in rate of filtration. While this clearance does not give an absolute measure of the amount of glomerular filtrate formed, it is safe to state that changes in urea clearance in any one individual are accompanied by changes in glomerular filtration in the same direction (32). Van Slyke, Rhoads, Hiller and Alving (33) found that changes in urea clearance were accompanied by parallel changes in renal blood flow. Bing (6) and Berglund and coworkers (4) have measured proteinuria in combination with creatinine clearance. The results were similar to ours.

Employment of a method giving a true measure of the amount of glomerular filtrate formed and simultaneous measurements of proteinuria would be necessary to establish the exact relationship between the two. Even then it is doubtful if one could expect more than qualitative changes in the same direction. Proteinuria varying in a manner

exactly proportional to the amount of filtrate would require that increase in permeability take place by uniform enlargement of the pores in the filtering membrane. As Richards et al. (30) have pointed out, it is likely that damage results in the formation of abnormal pores of varying size scattered throughout the glomeruli. These permit the escape of minute amounts of serum as well as occasional erythrocytes. Such irregularity of damage could explain the minor variations in proteinuria we have found from day to day in patients on a uniform regime, as well as the lack of uniformity in daily albumin:globulin ratios of the urinary proteins.

The preceding discussion does not include the possibility, suggested by Ekehorn (34), of serum protein being reabsorbed by the tubular epithelium. He advanced this possibility because smaller amounts of protein were found in the bladder urine of certain animals than could be explained from its concentration in the glomerular filtrate when the latter was obtained by direct puncture of the glomerular capsule. He assumed that the protein concentration of the particular glomerulus was representative of that in all the glomeruli of the kidney, a doubtful assumption under the conditions. Reabsorption of hemoglobin in the tubules has also been suggested by several investigators (34, 35). The basis for this suggestion was the finding of iron staining pigment in the tubule cells of patients with hemoglobinuria or animals which had been given hemoglobin intravenously. The splitting of the hemoglobin in the lumina of the tubules and subsequent absorption of the pigment derivative is equally likely, especially since some of the iron staining pigment has usually been found in the tubular lumina as well as in the epithelial cells.

#### SUMMARY

Simultaneous increases in proteinuria and urea clearance have been produced by increasing the protein of the diet, by administration of diuretics and by increasing the volume of the blood plasma. The latter effect was accomplished by transfusion of plasma. A somewhat higher concentration of plasma protein persisted for some time after readjustment of the plasma volume to the pretrans-

fusion level and was accompanied by loss of more protein in the urine.

Increases in proteinuria in Bright's disease may be explained by the presence of one or more of the following factors:

- (a) Increase in glomerular permeability.
- (b) Increase in the rate of glomerular filtration.
- (c) Presence in diet or in body reserves of more new material from which plasma proteins may be constructed.
- (d) Artificial increase in the concentration of plasma protein such as follows trans-fusion.

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# STUDIES IN THE PHYSIOLOGY OF BLOOD VESSELS IN MAN. APPARATUS AND METHODS. I. A SENSITIVE PLETHYS- MOSPHYGMOGRAPH FOR A PORTION OF THE FINGER<sup>1</sup>

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The apparatus I wish to describe was designed for the study of volume changes due to varying degrees of fullness of the smaller blood vessels in a portion of the human body, particularly for that portion of the finger distal to the major skin creases at the terminal finger joint.

The principal characteristics aimed at in devising the apparatus and method were: 1. Accurate measurement should be possible for either quick volume changes due to the pulse when as small as 0.5 cu. mm. and of slower changes in volume of relatively great magnitude, that is up to 800 cu. mm. 2. Each type of measurement should be based on graphic records made simultaneously. 3. In use, no influence of instrumental origin should modify the physiological phenomena under study, particularly by mechanical pressures causing constriction and consequent distension or emptying of blood vessels or by causing pain, fear, or other discomfort. The temperature, humidity and movement of the air around the skin should be normal. 4. The accuracy of measurements should not be impaired by change in room temperature. 5. The instrument should be dependable and easy to use. The apparatus about to be described seems to meet satisfactorily these requirements except those concerning skin environment and the effect of room temperature.

The apparatus, other than photokymograph and lamp, consists of a fine recorder (Figure 1), a coarse recorder and calibrator (Figure 4), a finger container cup and connecting tubes (Figure 5), all of which enclose a common space filled with air slightly above atmospheric pressure. The volume of the air space is susceptible to change by movements of the walls in only three separate portions of the apparatus, first by bulging or retraction of the walls (skin) of that portion of

the air space which comes in contact with the body part being observed; second, by change in shape and size of the fine recording capsule; third, by lengthening or shortening the metal bellows in the coarse recorder. Whenever the air space is encroached upon by a slight swelling of the finger, the recorder capsule bulges by an almost equal volume. The volume increase of the capsule is slightly less than the volume increase of the finger due to the compression of the air in order to overcome the resistance of the capsule to distortion. If the swelling of the body part is of great magnitude, great distension of the capsule tends to occur, but is prevented by the intervention of the operator who, being warned by the wide excursion of the light beam, brings the system back to original volume and pressure by accommodating the air displaced from the finger cup within the coarse recorder where the volume of the bellows is increased until the fine recorder has returned to its zero range. A beam of light from one of the coarse recorder mirrors traces a record of this large volume change.

The three principal units of the apparatus which encloses the air space are connected by three lengths of rubber tubing leading to the three arms of a four-way metal connection from the fourth arm of which another length of rubber tubing makes connection with a stopcock which opens or closes the air space. Each of three lengths of rubber tubing is one foot long, and the fourth, that which leads to the finger container cup, is six feet long. The internal diameter of all tubing and connections is  $\frac{1}{16}$  inch and the outside diameter  $\frac{3}{16}$  inch. A thin coating of stopcock grease was always applied to the metal connection parts before inserting them into the rubber tubing. Leaks, which have given little trouble, are indicated by drift of the fine recorder beam. Constancy in the volume of the enclosed air space is dependent upon constancy in temperature. A

<sup>1</sup> Aided by grants from The David Trautman Schwartz Fund, The Josiah Macy, Jr. Foundation, and The Committee on Scientific Research of the American Medical Association.

change in temperature of  $1^{\circ}\text{C}$ . produces a volume change of approximately 0.26 per cent, which for an air volume of 5 cc. would cause a volume change of 13 cu. mm. The apparatus has been used solely in a room where the temperature was kept automatically at either  $21.1^{\circ}\text{C}$ . or  $23.9^{\circ}\text{C}$ . with a maximum variation of  $\pm 0.3^{\circ}\text{C}$ . The apparatus functions to a certain extent as a radiometer, giving in response to hyperemia of the skin a beam deflection indicative of increased finger volume which is in part actually due to the increased temperature of the skin and the consequent increased temperature of the air within the finger container cup. However, the temperature effect is usually quite small as compared with the actual volume change of the finger.

*The fine recorder.* The fine recorder (Figure 1) is an optical capsule employing the well-known principles of the Frank segment capsule and differing chiefly in that stretching of the rubber membrane is largely avoided. Instead of stretching the rubber tambour over a rigid metal cup as is the usual procedure, a shallow flexible cup of thin rubber was used which permitted bulging of the membrane with less stretching. The capsule ( $C$  and  $C_f$ ) was made of two circles ( $\frac{7}{16}$  inch in diameter) of sheet rubber (made by the anode method, Miller Rubber Company, Akron, Ohio) 0.007 inch thick stuck together at their peripheries. In the finished capsule, the front circle carried the mirror ( $m$ ) and the rear circle which had been slightly cupped before being joined to the front circle, served to exert slight stretch on the front membrane. In making the capsule, the two small pieces of sheet rubber were vulcanized to each other by a heated dull cork borer. The piece which was to serve as a cup was cemented to the squarely cut end of a piece of copper tubing ( $t$ ) (outside diameter  $\frac{1}{8}$  inch) and the tube unsealed by inserting a hot wire into the lumen. The tube was slipped into a hole at the bottom of a shallow crater which had been turned in the surface of a small brass block. The piece of rubber was cupped by pressing the center to the desired depth in the crater. The front membrane was slightly stretched over the concave surface of the rear piece and the heated tube applied. At this stage all parts were held mechanically in proper alignment and the degree

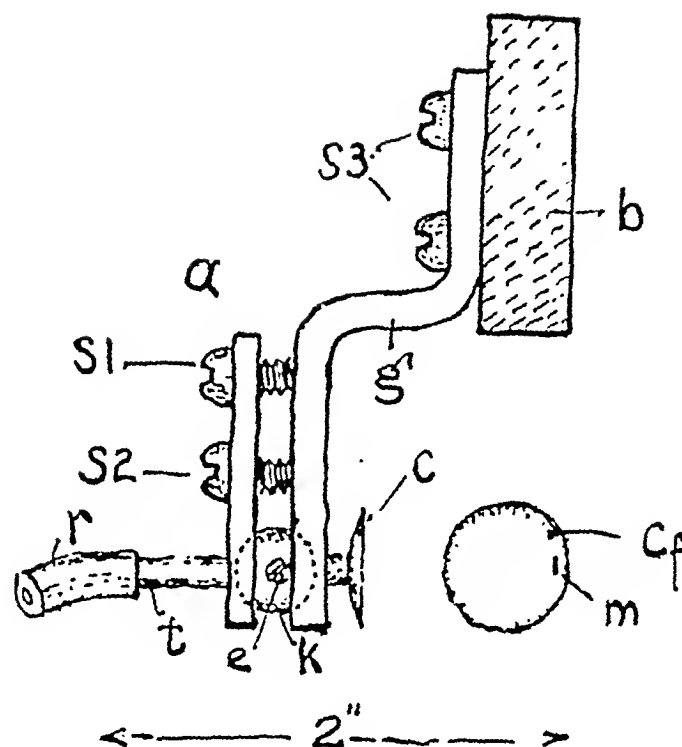


FIG. 1. FINE RECORDER CAPSULE UNIT

View from right side and in addition a front view of capsule ( $C_f$ ) and mirror ( $m$ ). Rigid brass bar ( $b$ ) in cross section upon which different recording devices are mounted. Screws ( $S3$ ) pass through vertical slots in part ( $g$ ) which allow adjustment vertically.  $C$  = capsule side view.  $t$  = copper tubing (outside diameter =  $\frac{1}{8}$  inch).  $K$  = ball through which tube passes; the ball fits into sockets in parts ( $a$ ) and ( $g$ ).  $e$  = setscrew with countersunk head.  $r$  = rubber tubing.  $S1$  = screw threaded into both ( $a$ ) and ( $g$ ) and serves as hinge for the two parts.  $S2$  = screw threaded only into ( $g$ ), adjustment of which tightens or loosens ball ( $K$ ) in socket permitting setting of filament image from mirror ( $m$ ) at proper natural zero, and also by rotation about axis of tube to adjustment of long axis of mirror to vertical.

of stretch of the front membrane controlled accurately by having it fastened to a metal ring through which, for the desired distance, could be thrust, by a screw mechanism, a smaller ring producing stretch of the desired degree. These parts as a unit were held in the lathe chuck, and the heated cork borer, its cutting edge accurately shaped, was carried by the tail piece of the lathe quickly and accurately through the opening of the inner ring. The two pieces of membrane were caught between the surface of the crater block and the edge of the heated tube and sealed to each other. Small leaks due to failure of vulcanization were sealed by passing them through a small drop of rubber cement two or three times with intervals

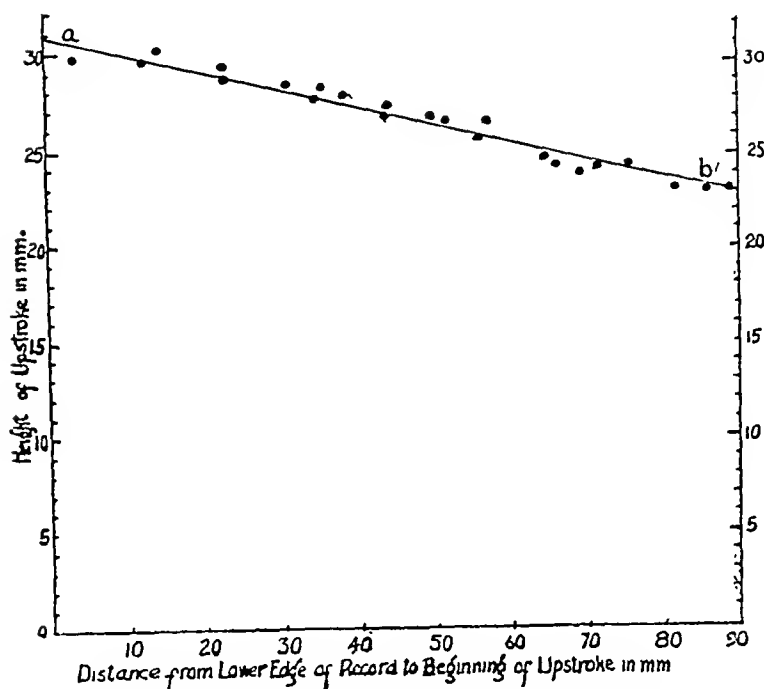


FIG. 2. VARIATION IN VOLUME RESPONSE OF THE FINE RECORDER DUE TO STATE OF FULLNESS OF THE CAPSULE

Data for the graph were obtained by making 69 quick calibrations in groups of three distributed across the width of the photographic strip. Height of upstroke refers to beam deviations caused by diminution of calibrator volume by 5 cu. mm., each dot representing the mean of 3 different deviations or upstrokes. The lower edge of the photographic record was to the right of the capsule during the process of recording. As the volume of the capsule increased the beam swung from right to left (upward on the finished record) and with increasing volume the size of the deviation per unit of volume change in the calibrator tended to diminish. Volume of the air space was approximately 7.0 cc. Owing to the fact that the natural zero point for the recording beam was at a point 40 mm. from the right edge of the photographic strip while in the camera, a slight degree of inflation of the capsule had been accomplished before the beam fell on the strip.

between for drying. A rectangular mirror (*m*)  $\frac{3}{64} \times \frac{1}{64}$  inch was stuck with shellac to the front membrane about  $\frac{1}{16}$  inch from the circumference with the long axis parallel to a chord of the circle. The capsule was mounted by a rigid support in front of the photokymograph, as shown in Figure 1. A straight filament lamp<sup>3</sup> was mounted rigidly  $4\frac{1}{2}$  inches in front of and slightly below the mirror with filament vertical and side of bulb toward mirror and completely enclosed in a metal

housing except for a narrow window which allowed the light to fall only upon the mirrors and the adjoining spaces. A photokymograph carrying bromide paper 120 mm. wide and driven vertically downward was placed  $21\frac{1}{2}$  inches in front of the mirrors.

Individual capsules differed somewhat in sensitivity, ranging from 3 to 9 mm. deflection for a volume change of 1 cu. mm. Deflections are read to the nearest 0.1 mm. on the record, which for a 5 to 1 calibration means reading to the nearest 0.05 cu. mm. Capsules of somewhat different design have had a sensitivity of 14 to 1. Such extreme sensitivity involves difficulties since small

<sup>2</sup> Westinghouse Electric & Manufacturing Company No. 594186.

<sup>3</sup> Westinghouse Electric & Manufacturing Company No. 463604.

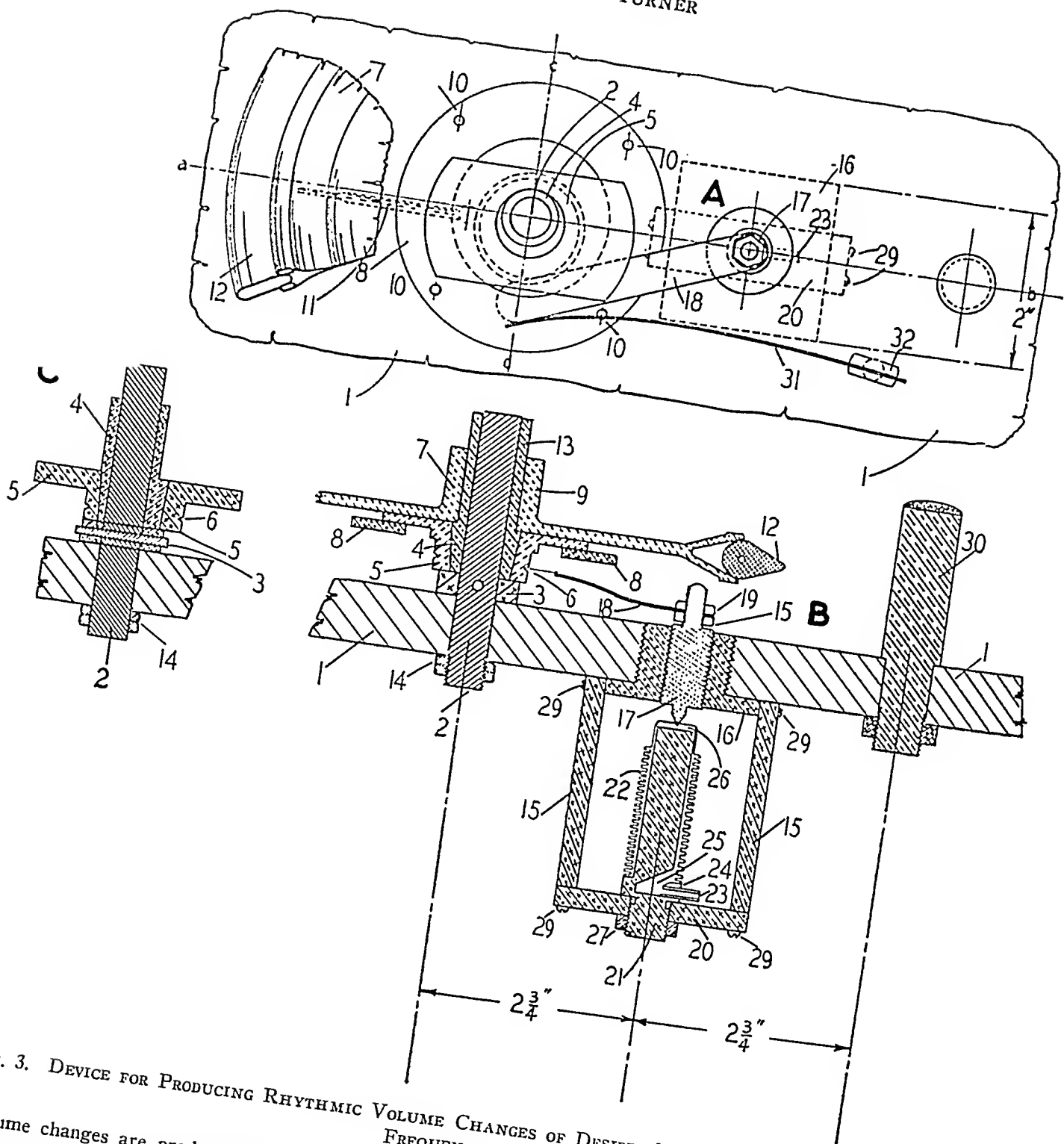


FIG. 3. DEVICE FOR PRODUCING RHYTHMIC VOLUME CHANGES OF DESIRED MAGNITUDE AND AT VARYING FREQUENCIES

Volume changes are produced by shortening or lengthening of the metal bellows (22) by the screw (17) which is rotated back and forth through a variable angle maximum at 3° by the lever (18) which rests in a circular v-shaped groove on the face of the cylinder (6) which functions as a variable eccentric which may be set so as to impart to lever (18) at point of contact a to-and-fro movement of from 0 to 1/4 inch. For the tests mentioned in the accompanying article the apparatus was driven directly through a short piece of rubber tubing, one end of which was slipped over the motor shaft and a shaft-like projection bolted to the hub of the wheel (7).

Three partial views:

- A. Top view with all except a fragment of drive wheel (7) and rubber tire (12) cut away.
- B. Partial sectional view through a-b.
- C. Sectional view of eccentric assembly through c-d.



volume changes are likely to cause the beam to leave the photographic film.

The degree of proportionality between volume change and deflection of the recording beam throughout the recording range is shown in Figure 2. The deflections due to both the calibrator and pulse are routinely recorded so as to be centered upon the same longitudinal line on the photographic strip. Recording pulse volumes of greater or less amplitude than that of the calibration introduces a positive error on one side of the line and a negative error on the other which tend to neutralize each other. The tendency toward diminished deflection per unit volume change as the capsule volume increases is accentuated as the

volume of the total air space of the apparatus increased, but is of little importance for volumes of less than 10 cc. Air pressures within the capsule, as we have used it, do not exceed 12 mm. of water above atmospheric pressure.

The maximum length of life of these capsules has not been determined. However, one has been in regular use for eleven months without evidence of deterioration.

So far as I am aware, no one has established such comprehensive criteria for the volume-frequency characteristics of a capsule designed for recording volume changes with minimal pressure in the system as exist for pressure recording in which volume changes are kept as low as possible.

All parts made of brass unless otherwise specified.

1. Steel base plate.
2. Steel shaft.
3. Cuff fastened to shaft with tapered steel pin.
4. Sleeve which acts as bearing for pulley; upper portion concentric with shaft and lower portion concentric with center  $\frac{1}{16}$  inch off that of shaft. This is the inner eccentric.
5. Outer eccentric with peripheral circle centered  $\frac{1}{16}$  inch from that of inner eccentric. By rotation of outer eccentric upon inner eccentric the distance between the centers of the shaft (2) and the outer eccentric may be varied from 0 to  $\frac{1}{8}$  inch. The circumference of the outer eccentric may impart a maximum motion of  $\frac{1}{4}$  inch along the radius of a circle centered with shaft (2) to an object pressed against it.
6. Groove in outer eccentric in which rocker arm (18) rests.
7. Aluminum V-pulley fixed to (3) by 4 screws, one of which is (9).
8. Clamping ring. By tightening 4 screws (10) the outer eccentric is fixed to pulley (7) by friction, holding a given setting of outer eccentric indicated by pointer (11) on graduated scale on rim of pulley. This scale is graduated in cu. mm. volume change of bellows.
9. Setscrew.
10. Holes for screws.
11. Steel pointer for indicating on scale volume change in bellows per revolution of pulley.
12. Solid rubber tire which establishes driving contact with brass drum on motor shaft. (Motor and driving drum not shown.)
13. Cuff for holding moving parts on shaft.
14. Nuts.
15. Lateral pieces of bellows frame.
16. Top piece of bellows frame fixed by screwing into base (1) and receiving the screw (17).
17. Screw with threads 16 per inch,  $\frac{1}{4}$  inch pitch, lead of 4, rotated back and forth by rocker arm (18) through an arc up to  $3^\circ$  as determined by the setting of outer eccentric (5).
18. Rocker arm of phosphor bronze  $\frac{1}{16}$  inch thick.
19. Nuts.
20. Bottom piece of bellows frame through which bellows filler (21) is threaded and held by nuts (27).
21. Bellows filler for limiting dead space.
22. Metal bellows (Fulton-Sylphon, Number 107611-24B).
23. Piece of  $\frac{1}{8}$  inch copper tubing soldered into hole (24) connecting with hole (25) establishing connection between the air space (26) within bellows (22) and the connecting tube.
24. Hole.
25. Hole.
26. Air space.
27. Nuts.
28. Screws which hold together the 4 parts of bellows frame.
29. Upright shaft upon which is mounted driving motor.
30. Spring  $\frac{1}{32}$  inch thick of phosphor bronze  $\frac{1}{2}$  inch wide. Keeps rocker arm (18) pressed snugly into groove on the outer eccentric.
31. Spring holder. The lower portion.

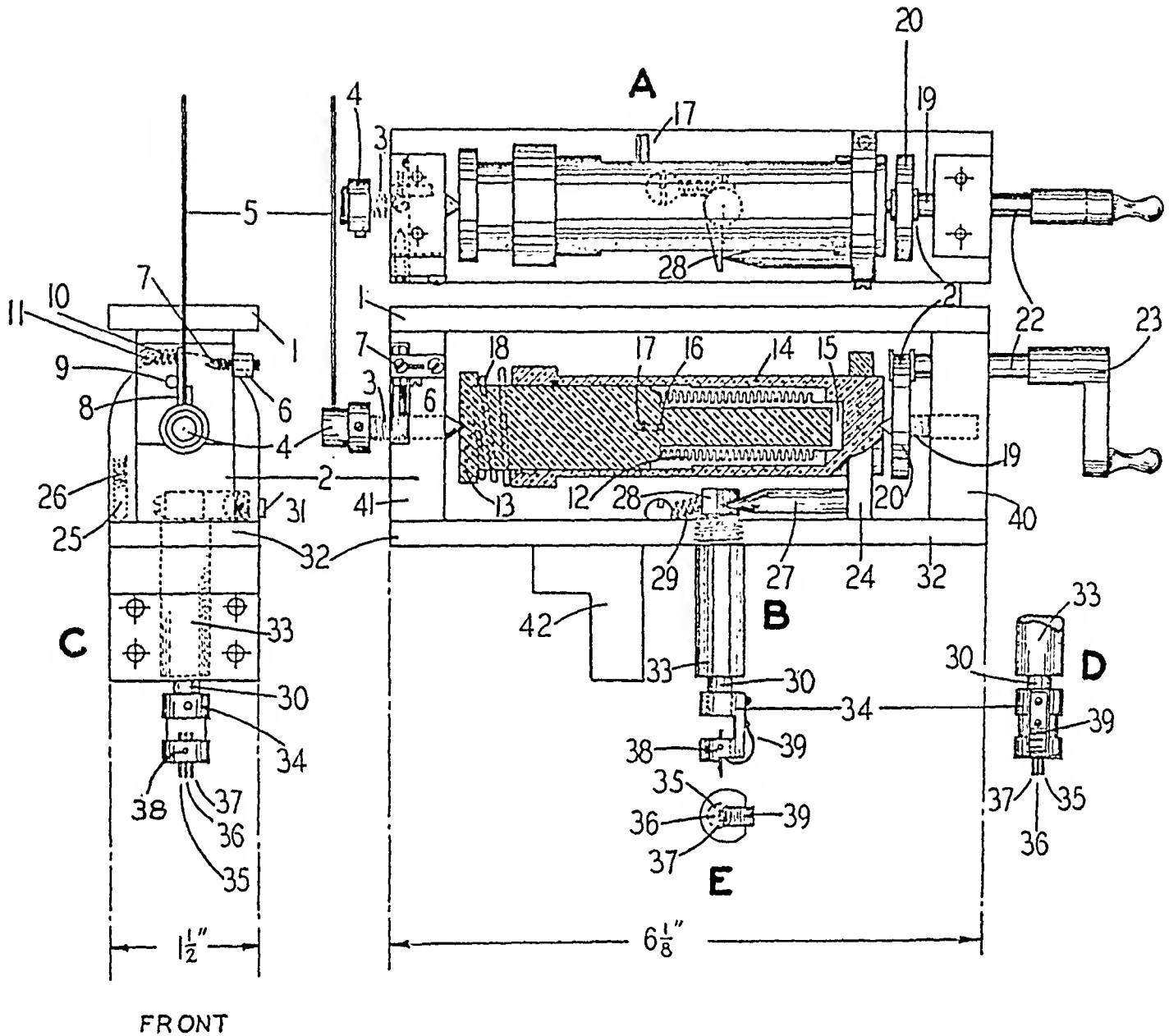


FIG. 4. THE COARSE RECORDER AND QUICK CALIBRATOR

- A. View from above with top horizontal, (1) removed and quick calibrator level (5) and portion of cuff (4) cut away.
- B. View from left side showing in section the bellows (12) and the male (13) and female (14) parts into which the two ends of the bellows are soldered.
- C. View from the front.
- D. View from rear showing only the mirror shaft assembly.
- E. View of mirror assembly only, from below.

All parts made of brass unless otherwise specified.

1. Top piece of frame.
2. Front end piece of frame.
3. Screw, 20 threads per inch, for instantaneous calibrator.
4. Cuff.
5. Calibrator handle of number 18 music wire.
6. Split block for screw (7).
7. Adjustable stop of steel for instantaneous calibrator.
8. Steel lever passing through screw (3), movement limited by adjustable stop (7) and fixed stop (9).
10. Spring attached between steel lever (8) and screw (11).
12. Brass bellows (Fulton-Sylphon Number 107611-24B) length varied by the movement upon each other of two

sible. It has seemed advisable to observe the response of the capsule to known equal changes in volume of the air space in another portion of the apparatus at widely varying frequencies. For the purpose of bringing about these volume changes at the desired frequencies it was necessary to design and build a special device. The construction and mode of operation are shown in Figure 3 and the accompanying legend. The capsule has been tested by joining the variable air space of this apparatus to the air spaces of the capsule and calibrator by means of the same rubber tubing used for connecting the finger cup. For each test equal rhythmic volume changes were employed at varying frequencies. In different tests the volume change varied from 2 to 10 cu. mm. In a typical experiment using volume changes of constant size and at increasing frequencies, it was observed that at 5 cycles per second the amplitude recorded by the deflected light beam was 19.5 mm. which increased with increasing frequencies so that at 10 cycles per second the amplitude was 23.5 mm. where it maintained a high degree of constancy up to a frequency of 40 cycles per second when the amplitude of the deflections began to diminish and at 60 cycles per

second had fallen to an amplitude of 12 mm. The chief purpose for which this recorder was designed was the measurement of the volume changes due to the pulse in a portion of the finger. The most difficult task for such a device is the recording of the upstroke of the pulse wave which may occupy as little as 0.01 second, the equivalent of half of a 50 cycle wave. The behavior of the capsule was observed, using several technics, when it was required to make one quick change in volume. In one technic the capsule was connected to one of two short pieces of rubber tubing which were separated by a glass stopcock. The open end of the second piece of tubing was closed by a clamp and with the stopcock closed either positive or negative pressure was produced within the second piece of tubing by the use of another clamp. When the stopcock was quickly opened, there was a single quick deviation of the recorder beam without overshooting to a new position typically as follows. The entire deviation of the filament image was 37 mm., 92 per cent (34 mm.) occurred in 0.016 second and 8 per cent (3 mm.) required 0.080 second. This is the behavior of a highly damped recorder. Using another technic, when a deviation of 55 mm. oc-

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parts (13 and 14) holding the two ends of bellows.

13. Male part and dead space filler soldered into open end of bellows.

14. Female part into which closed end of bellows was soldered.

15. Air space varied by length of bellows.

16. Hole leading from air space to rubber tubing slipped over tube (17).

18. Spring which tends to lengthen bellows, thrusting male part against screw (3) and female part against screw (19, 80 threads per inch, left handed) which is rotated by gear (20, 32 teeth) in mesh with gear (21, 6 teeth) on shaft (22) which is turned by handle (23).

24. Part soldered to (14) prevents rotation of bellows supports by sliding along bottom of frame (32) against which it is tightly pressed by pin (25) and spring (26).

27. Rod with eccentric point held in part (24) by screw (31); point presses against flat face of lever (28) which rotates shaft (30) which carries coarse recorder mirror. Lever 28 is held against point of rod (27) by spring (29).

33. Bearing for shaft (30). Cuff about 30 preventing upward movement of shaft not shown in drawing.

34. Mirror carrier block.

35, 36, 37. Mirror carrier rods of number 5 jewelers' pivot steel, lower ends cut at an angle of  $7^\circ$  from axis of shaft (30) to receive mirrors.

38. Setscrew for fixing mirror rod (36).

39. Flat spring split to make 3 springs which prevent mirror carrier rods from falling out where screws are loosened.

40. Rear frame end piece.

41. Front frame end piece. Same as (2).

42. Bracket for fastening instrument to supporting horizontal bar by means of screws. The same bar also supports the fine recorder capsule. Both coarse and fine recorder mirrors (Westinghouse number 594186' receive light from same straight filament lamp (Westinghouse number 463604) from a distance of  $4\frac{1}{4}$  inches and both record on the same moving strip of bromide paper at a distance of  $21\frac{1}{2}$  inches without the use of lens except the cylindrical lens of the photokymograph.

curred in less than 0.002 second there was some overshooting. In routine use the regular mode of quick calibration gives a volume change requiring 0.033 second which is the equivalent of one-half cycle at a frequency of 15 per second.

*The coarse recorder.* This unit consists of the coarse, or slow, recorder and the quick calibrator, the construction of which is shown in Figure 4 and the accompanying legend. The rods (35, 36, 37) supporting the mirrors are so adjusted as to space evenly the three images of the lamp filament at such distances apart that, as the mirror shaft (*A, B, C* 30) is rotated, one image does not leave the photographic record before the next arrives upon it. In this way the coarse recorder utilizes the equivalent of a photographic strip slightly less than three times the width of that actually employed. By rotating the rod with the eccentric point (*A* and *B* 27) the effective length of the lever (*A* 28) can be varied so that the value in cu. mm. of volume of a given deviation of an image on the photographic record can be set at a convenient figure. For calibrating the coarse recorder the outlet was connected by rubber tubing to a pipette of 0.1 cc. capacity which could be read to 1 cu. mm., clamped in a horizontal position and containing a small drop of alcohol. By turning the crank (*A* and *B* 23) volume change in the metal bellows (*B* 12) was produced, displacing the drop along the lumen of the pipette. By changing the volume in units of 10 cu. mm. and stopping the meniscus of the drop for a moment at each 10 cu. mm. mark on the pipette, a step-like line was produced on the record. The lateral displacement of the line at each step represented a record of a volume change of 10 cu. mm. by that particular mirror and position on the photographic strip. By opening the system and putting the drop back to the starting end of the pipette such equal changes in volume were recorded with the image from each of the three mirrors for the entire width of photographic paper. No significant variation in calibration factor was found throughout the recording range. The setting routinely used gives 1 mm. deflection of the filament image for a volume change of 0.18 cu. mm., and the records are measured to the nearest 0.1 mm. The photokymograph used was made in our laboratory by Dr. W. H. Gillentine

to whom I am indebted. The cylindrical lens is graduated in millimeters so that the developed record is ruled longitudinally in parallel lines 1 mm. apart as is customary for electrocardiographic records. These lines are not photographed unless illumination is provided for that purpose. A small four candle power bulb is arranged in a housing alongside the capsule so as to provide sufficient light over the entire width of bromide paper to produce a shade of gray which contrasts with the white parallel lines and the black lines due to the filament images reflected from the mirrors.

*The quick calibrator.* Since the sensitivity of the fine recorder varies slightly with the volume of the enclosed air space in the apparatus, it is essential that its response to known volume change be determined each time the apparatus is newly applied to the study of a finger or whenever there is likelihood that volume change in the closed system of disturbing size has occurred. In most instances the enclosed air space has a volume of from 4 to 6 cc., and there is no striking change in calibration until the volume exceeds 10 cc. Calibration is accomplished by striking the spring handle (*B* and *C* 5 in Figure 4) of the quick calibrator with the finger so that the screw (*A* and *B* 3) is rotated as far as stop (*C* 7) will allow when the bending of the handle allows the finger to continue its swing releasing the mechanism and allowing it to return to its original state. In this way the bellows is quickly compressed giving a diminution in volume, then following a brief pause it is quickly returned to its original volume. The volume of the sensitive recorder capsule is suddenly increased then diminished by this procedure and a photographic record of the deviations of the filament image reflected from the capsule mirror provides the standard unit for determining what volume changes are represented by pulse complexes recorded by the same capsule. The calibration value is adjusted to a desired figure by the setting of the screw (7 *C*) as determined by the movement of the alcohol drop when the calibration technic is executed slowly. We have used routinely a volume change of 5 cu. mm.

*The finger container cup.* It is extremely difficult to enclose a sharply defined portion of the human body in a rigid container so that pressure

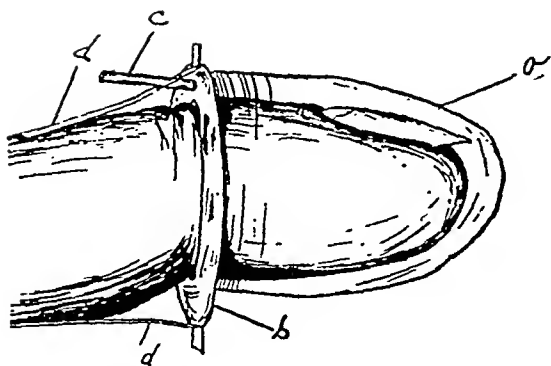


FIG. 5

*a. Metal cup:* In making the metal cup, 1 × 6 inches standard (tin-coated) collapsible metal barrels (Standard Specialty and Tube Company, New Brighton, Pa.), such as used for toothpaste tubes, are cut into desired lengths and molded over carved wooden positive molds of sizes varying so that the internal surface of the cup shall be about 2 mm. from the surfaces of the enclosed portion of the finger. After shaping the cup, the creases and cracks are sealed with cement (DuPont Household Cement).

*b. Diaphragm:* The diaphragm is cut into shape out of transparent Pyralin sheets (DuPont Viscoloid Co., Arlington, N. J.), 0.030 inch thick. A 1.5 mm. opening is made for the continuation of the bore of the brass tube and about a 4 mm. opening is made in the center. The latter opening is subsequently enlarged and properly shaped to fit the subject's finger.

*c. Brass tube:* The tube, approximately 2 mm. external diameter, is made of sheet brass, 0.002 inch thick, with a soldered seam.

*d. Cellulose acetate strips,* approximately 0.25 inch wide and 0.008 inch thick for supporting the cup on the finger.

on, or tugging at the skin sufficient to disturb filling of small blood vessels does not occur. The terminal portion of the human finger has sharply defined landmarks and lends itself to a high degree of isolation and the blood supply is rich and varies widely under physiological and pathological influences. The cup which encloses the finger part should be light in weight and enclose a minimum air space which conforms exactly to the anatomical landmarks. The seal should be airtight and at the same time conform so easily to skin surfaces, even though the finger swell or shrink after the seal is applied, as to exert no disturbing pressure or tugging. The cup shown in Figure 5 seems to meet these requirements. I am indebted to Dr. G. E. Burch, Jr., who has

made this unit a satisfactory part of the apparatus. The construction is explained in the legend for Figure 5. Several completely assembled units of different sizes, needing only shaping of the hole in the diaphragm to fit the finger, were kept available. When a subject presented himself for study the proper size cup was chosen and the opening in the diaphragm carefully shaped so that it fitted the finger loosely at the major dorsal and palmar creases at the terminal interphalangeal joint when the finger was comfortably flexed. The supports (*d*) were adjusted so that they rested upon the finger without exerting any significant pressure or tugging and were stuck in place with rubber cement. The junction between the skin and the diaphragm was sealed air-tight with a non-constricting, elastic, adhesive composition which had been previously heated to body temperature. The composition consists of two parts of printers' roller compound and one part of a water-soluble, non-irritating, lubricating jelly.<sup>4</sup> The rubber tubing leading to the recording apparatus is fixed to the dorsum of the subject's hand with adhesive tape, some slack being allowed between the cup and the adhesive tape. The complete finger unit weighs from 2 to 7 grams and contains about 3.5 cc. of air-space around the enclosed portion of the finger. Turning the finger gently so as to reverse the direction of the force of gravity causes no significant volume changes. Sudden movements of the fingers do cause quick transitory deflections of the fine recorder light beam. Unsatisfactory features of the finger container cup are that the air about the finger moves little, becomes heated, and soon develops high humidity, and unless the room is comfortably cool the sweat collecting in the air space may reach, in long experiments, an appreciable volume.

#### SUMMARY

An apparatus designed particularly for measuring volume changes due to the state of fullness of the blood vessels of a sharply defined portion of the human finger is described as to construction, mode of operation and working characteristics. The apparatus makes a graphic record of

<sup>4</sup>K-Y Lubricating Jelly, Johnson and Johnson, New Brunswick, N. J.

pulse volumes as small as 0.1 cu. mm. and of gradual volume changes as great as 1000 cu. mm. The pulse recorder which employs an optical capsule in which stretching of the rubber membrane is largely avoided shows high sensitivity, low moving mass, responds well to volume changes at a frequency up to 40 cycles per second and with diminished amplitude to 60 cycles per second and is well damped. The apparatus and method interfere with the body part under study to a minimal extent except through undesirable tempera-

ture and humidity of the air in contact with the skin.

*Acknowledgments.* I am indebted to Mr. George Johnson who constructed the apparatus and shared in all manipulations, and to my associates Drs. W. A. Sode-man and G. E. Burch, Jr., for valuable aid of many kinds.

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# STUDIES IN THE PHYSIOLOGY OF BLOOD VESSELS IN MAN. APPARATUS AND METHODS. II. A METHOD FOR THE DETERMINATION OF THE VOLUME OF THE SOFT TISSUE ABOUT THE TERMINAL PHALANX OF THE HUMAN FINGER<sup>1</sup>

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For quantitative studies in the physiology of the peripheral blood vessels of the human which are under way in this laboratory the soft tissues of the terminal part of the finger have been found particularly satisfactory. This communication deals with a method for estimating in the living human the volume of soft tissues in that portion of the finger which, for lack of a better term, we call the finger tip.

The method consists of a determination of the total volume of the finger tip, an estimation of the bone volume and a calculation of the soft tissue volume by difference. The finger nail was included in the soft tissue volume.

The finger tip has been defined, in terms of skin markings, as that portion of the finger distal to a plane passing through the center of the dorsal and palmar skin crease at the distal interphalangeal joint. This plane passes through the distal end of the second phalanx.

The total volume of this portion of the finger, when held at heart level, was determined by making a cast of the part in dental stone.<sup>2</sup> The setting expansion of the dental stone is only 0.13 per cent. The mixture, as a free flowing fluid, moulded itself about the finger without deforming pressure and accurately reproduced minute details of skin surface. The open end of the cast was then ground down to the desired plane with a file and emery cloth and overfilled with mercury. By pressing a glass plate firmly over the open end of the cast the excess mercury was forced to escape. The remaining mercury was then transferred to a microburette by means of a pipette, and measured to one cubic millimeter.

Repeated determinations of the volume of one finger tip over a period of two days with sixteen independently made casts gave volumes varying from 4.398 to 4.492 cc. Statistical analysis of these sixteen trials gave a mean of  $4.453 \pm 0.005$  cc., a standard deviation of 0.027 and a probable error of 0.018.

To determine bone volume a formula was derived from data on thirty-three sets of phalanges covering the range of sizes seen in our subjects. Phalangeal bones of the second, third and fourth fingers were mounted upon wooden blocks with paraffin to simulate the bony anatomical relationships in the living subject. A level corresponding to the plane of the skin markings was laid off with radiopaque foil and anteroposterior and lateral roentgenograms taken at a tube distance of six feet. From these plates the areas of the segments of the bone shadows desired were obtained with a planimeter. The joint space was included in the measured area. Its borders were determined in the anteroposterior view from anatomical markings clearly shown on the roentgenogram. In the lateral view, because of the lack of anatomical landmarks, projections of the outline of the terminal phalanx to the shadow of the second phalanx were used. Lengths of these segments were determined to 0.1 mm. by use of a caliper, millimeter scale, and lens. By dividing lateral area by length a figure termed the "mean thickness" resulted. Anteroposterior area multiplied by mean thickness resulted in a trial calculated volume.

True volumes of the bones were determined by use of dental stone casts in a manner similar to that used for total finger volume. In these determinations, however, split casts were used. The thirty-three volumes varied from 0.341 to 1.183 cc. Percentage differences between the measured volumes and the trial calculations were deter-

<sup>1</sup> Aided by grants from The David Trautman Schwartz Fund, The Josiah Macy, Jr. Foundation, and The Committee on Scientific Research of the American Medical Association.

<sup>2</sup> The proprietary product Investone was used.

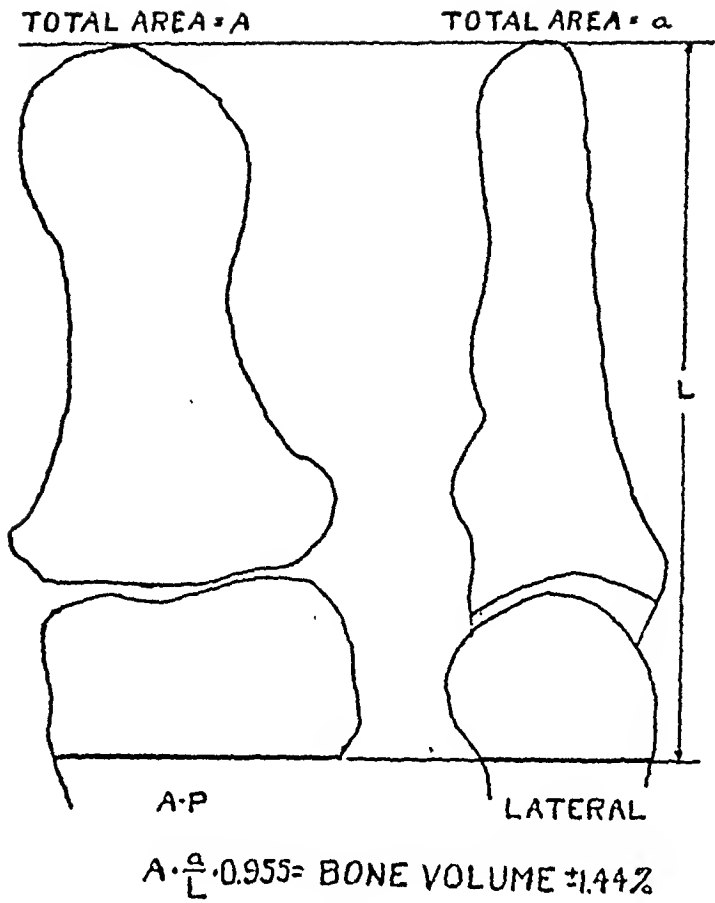


FIG. 1.

DIAGRAM ILLUSTRATING THE BONE SEGMENTS MEASURED IN THE DETERMINATION OF THE BONE VOLUME OF THE FINGER TIP

mined in each case and these differences analyzed statistically. Differences varied from 0.0 per cent to 8.6 per cent, the calculations being the higher values. Mean difference was  $4.50 \pm 0.24$  per cent, the probable error 1.44, and the standard deviation 2.12 for the frequency distribution. Application of these differences to a formula for

bone volume resulted in the following relationship:  $0.955 \times A \times \frac{a}{L} = \text{volume} \pm 1.44 \text{ per cent}$  where  $A = AP \text{ area}$ ,  $a = \text{lateral area}$ , and  $L = \text{length}$ .

In applying this formula to the living finger one must obtain accurate views of the finger with the aid of a support to hold the proximal plane of the finger tip parallel to the x-rays. For this purpose a metal support or shelf was constructed to hold comfortably the partially flexed hand with the finger to be x-rayed extending over the shelf to free it from deforming pressure and its axis perpendicular to the rays six feet from the tube. The x-ray film is held in a rigid support immediately behind the finger. In lateral views taken in this manner the soft tissues are well outlined and the skin creases used to define the limits of the finger tip easily identified. A straight line connecting the dorsal and palmar crease then delimits the bone segment to be measured. The length of this segment may then be determined with a dividers and laid off upon the bone segment of an *AP* view obtained in a similar manner.

TABLE I  
*Representative determinations on five subjects*

Subject	A	a	L	T	Calculated bone volume	Determined total volume	Soft tissue volume (Total volume minus bone volume)
	sq. mm.	sq. mm.	mm.	mm.	cc.	cc.	cc.
T	179.3	109.6	22.7	4.83	0.827	5.634	4.807
O	113.5	79.0	18.1	4.36	0.472	3.501	3.029
J	165.7	99.9	22.6	4.42	0.069	4.667	3.968
S	128.3	78.7	18.6	4.23	0.518	4.453	3.935
B	142.5	90.9	20.7	4.39	0.598	4.466	3.868



# STUDIES IN THE PHYSIOLOGY OF BLOOD VESSELS IN MAN. III. SOME EFFECTS OF RAISING AND LOWERING THE ARM UPON THE PULSE VOLUME AND BLOOD VOLUME OF THE HU- MAN FINGER TIP IN HEALTH AND IN CERTAIN DISEASES OF THE BLOOD VESSELS<sup>1</sup>

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(Received for publication May 4, 1937)

This investigation was carried out to throw some light upon the immediate responses of normal and diseased blood vessels to changes in level of the upper extremity. Observations were limited to reactions in the finger tip, defined as that portion of the finger distal to a plane passing through the major dorsal and palmar skin creases at the terminal interphalangeal joint. While the literature abounds in qualitative studies of this nature, quantitative data are rarely encountered. Determinations were made on the effect of passive elevation and depression of the arm upon the volume of pulsations and total blood volume in the finger tip of normal subjects and those with arteriosclerosis, diastolic hypertension, arteriosclerosis and diastolic hypertension and clubbing of the fingers.

## METHODS

Seventy-eight determinations were made upon 70 subjects, comprising 33 normal individuals from 12 to 65 years of age, and 37 abnormal subjects divided as follows: 13 with arteriosclerosis, 7 with diastolic hypertension, 10 with arteriosclerosis and diastolic hypertension, 2 with congenital clubbing of the fingers, 3 with acquired clubbing of the fingers, one with Raynaud's phenomenon (dead finger), and one with syphilitic aortitis with aortic regurgitation.

To eliminate the influence of menstruation and menopause, male subjects only were used. Observations were made under controlled atmospheric conditions (70° F. in winter, 75° F. in summer, relative humidity 50 per cent). After the subjects had rested in the sitting position for 30 minutes, metabolic rates were determined by the Benedict-Roth method. No dietary restrictions were made. Pulse rates and oral temperature were taken and subjects with fever eliminated. The sphygmoplethysmograph developed in our laboratory (1) was employed for the right index finger. Brachial blood pressure was

determined with the mercury manometer by the auscultatory method in the opposite arm. Care was taken to eliminate constricting influences upon the circulation, the subject, however, being comfortably clothed.

The subject was seated comfortably in a chair with the right forearm resting passively in a position midway between pronation and supination upon a support as if upon the arm of a chair. The support was so constructed that movement up and down took place about a fixed point as a center which coincided with the center of movement of the shoulder joint. With motion only at the shoulder joint the arm was then raised or lowered passively to a finger tip level 45 cm. above or below heart level. A continuous record was then made of the pulsations and other volume changes as follows: heart level, 30 seconds; lowered position, 30 seconds; heart level, 60 seconds; elevated position, 60 seconds; circulation occluded in the elevated position, 30 seconds; occlusion released and heart level, 60 seconds; lowered position, 60 seconds; and finally heart level, 60 seconds. Sudden occlusion of the circulation in the elevated position was accomplished suddenly with the use of a blood pressure cuff applied to the upper arm and connected to a 12 liter reservoir under pressure exceeding the systolic pressure of the subject by at least 50 mm. of mercury. At this time the part studied reached its minimum volume and for the purpose of our calculations the finger tip was assumed to be bloodless. This is not strictly true. Any given volume above this base line was accepted as representing the total blood volume of the part at that stage of the observation. The volume of the bloodless soft tissue was determined by subtracting the total blood volume at heart level by a method previously described (2). Throughout these procedures efforts were made to keep psychic influences at a minimum. The records were analyzed for volume of pulsations and total change in volume of the finger tip at each level.

## RESULTS

In all subjects there was a change of pulse volume with change in position. The pulse volume increased in the elevated position and decreased in the lowered position. With few exceptions, total blood volume varied in the inverse direction to the pulse volume. Table I shows the mean,

<sup>1</sup> Aided by grants from The David Trautman Schwartz Fund, The Josiah Macy, Jr. Foundation, and The Committee on Scientific Research of the American Medical Association.

TABLE I  
Maximum, minimum and mean values for total volume of blood, total blood volume as per cent of soft tissue, and volume of pulsation

	Normals			Arteriosclerosis			Diastolic hypertension			Diastolic hypertension and arteriosclerosis			Raynaud's phenomenon (dead finger)		Acquired clubbing			Congenital clubbing			Lucetic aortic regurgitation, (one case)
	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Affected	Normal	Maximum	Minimum	Mean	Maximum	Minimum	Mean	
ELEVATED POSITION																					
Total blood volume, cu. mm....	191.1	4.3	56.2	193.4	5.1	54.4	104.6	11.6	53.3	85.5	19.8	57.2	52.5	70.8	125.9	46.2	76.0	66.5	11.6	39.0	62.7
Total blood volume, per cent of soft tissue.....	4.08	0.12	1.38	2.77	0.12	0.98	2.36	0.31	1.17	1.82	0.52	1.30	1.23	1.26	4.27	0.86	2.06	1.15	0.31	0.73	1.34
Volume pulsation, cu. mm.....	21.5	0.5	4.6	17.4	0.7	5.3	6.3	0.4	3.6	5.8	1.6	3.3	2.9	4.6	3.8	1.2	2.3	0.8	0.4	0.6	22.0
HEART LEVEL																					
Total blood volume, cu. mm....	306.9	8.4	91.0	188.3	6.9	60.6	103.9	37.4	103.9	312.1	21.4	117.0	70.4	118.0	234.0	108.6	150.6	69.8	37.4	53.6	106.5
Total blood volume, per cent of soft tissue.....	6.48	0.43	2.16	2.70	0.12	1.09	2.34	0.94	1.63	6.86	0.51	2.49	1.65	2.75	7.94	1.69	4.03	1.32	0.99	1.16	2.29
Volume pulsation, cu. mm.....	7.9	0.4	2.8	7.4	0.6	3.6	4.5	0.5	2.4	4.5	0.8	2.2	2.4	2.6	4.2	1.2	2.5	0.8	0.5	0.65	10.6
DEPRESSED POSITION																					
Total blood volume, cu. mm....	460.3	52.8	192.2	215.9	30.5	127.8	243.6	25.1	136.5	199.9	22.7	130.5	75.8	153.7	260.8	160.6	201.2	125.4	121.2	123.3	128.8
Total blood volume, per cent of soft tissue.....	9.72	1.19	4.59	4.70	0.65	2.54	5.48	0.61	2.99	4.69	0.60	2.94	1.78	2.74	8.84	2.82	4.77	3.32	2.10	2.71	2.73
Volume pulsation, cu. mm.....	3.4	0.3	1.3	3.5	0.7	1.8	2.5	0.4	1.3	2.4	0.5	1.4	1.1	1.7	1.6	0.9	1.3	0.9	0.4	0.7	5.5

maximum and minimum values for the volume of pulsations, blood volume for the whole finger tip and as per cent of soft tissue at each level for the normal and abnormal groups.

In 39 observations on 33 normal subjects, the mean blood volumes at heart level and in the elevated and lowered positions were  $90.9 \pm 6.1$ ,  $57.6 \pm 4.4$  and  $191.6 \pm 8.8$  cu. mm. respectively. The volumes of pulsation were  $2.97 \pm 0.20$ ,  $4.68 \pm 0.42$  and  $1.30 \pm 0.08$  cu. mm. respectively. In subjects with arteriosclerosis without diastolic hypertension, the mean volume of pulsation was approximately that of the normal, and the mean total blood volume was reduced.

In subjects with diastolic hypertension, the volume of pulsations and total blood volume at heart level were reduced. In the elevated position the total blood volume was approximately that of the normal group but the volume of pulsations was again reduced. In the lowered position, the total blood volume was reduced while the volume of pulsation was that of the normal group. The range of individual values for the volume of pulsations and total blood volume was greatly limited in all positions as compared to the normal and arteriosclerotic groups.

In subjects with arteriosclerosis and diastolic hypertension the mean volume of pulsations and the total blood volume approximated closely the values obtained in subjects with diastolic hypertension alone.

In a subject with localized Raynaud's phenomenon (dead finger) with marked atrophic changes in the left index (affected) finger, distinct changes were noted at room temperature as compared to the corresponding normal (right index) finger. The involved finger showed a reduced total blood volume in all positions out of proportion to the reduction in total soft tissue volume. The volume of pulsations was slightly reduced at heart level and definitely so at the elevated and lowered positions.

In two cases of congenital clubbing of the fingers, the volume of pulsations was markedly reduced in all positions and was affected negligibly by position. The total blood volume was relatively and absolutely reduced in all positions as compared to all other groups. In patients with acquired clubbing of the fingers, values more

nearly approximated normal. However, the volume of pulsations in all positions was less than in the normal while the total blood volume was greater.

One subject with syphilitic aortitis with aortic regurgitation was studied. While blood volume in all positions approximated the normal, the volume of pulsations was greater than the maximum values for the normal group.

No correlation between the observed values and pulse rate, blood pressure (systolic, diastolic or pulse pressure) or metabolic rate was found.

The results for the various groups expressed in terms of soft tissue volume indicate that the differences were not due to variations in finger size, but to actual changes in relationship of blood to tissue volume.

#### DISCUSSION

We have utilized in the interpretation of our data on volume change of the finger tip some observations with a recording method (3) for measuring change in light reflection from the skin of the finger pad. Its use shows that the finger tip reflects more light in the elevated and less in the depressed position than at heart level and, furthermore, furnishes sphygmograms similar in form to the volume sphygmograms. They also show similar variation in amplitude with change in position. We interpret these observations as proof that the pulse wave is manifested by volume changes in the vessels which contribute most to skin color, and that these vessels are dilated in the depressed and narrowed in the elevated position. According to Lewis (4) these vessels are principally venules and to a less extent capillaries. What is known concerning the distensibility of veins indicates that all the veins of the part share in this change in caliber. The veins leaving the finger tip have an internal pressure of more than 35 mm. of mercury for the depressed position and only slightly above zero for the elevated position. We are unable to explain some of our observations on any other basis than that increase in total blood volume of the finger in the depressed position and diminution in the elevated position were due to change in caliber in veins and in spite of changes in the opposite direction by the arterial vessels. Any other explanation would also presume the lack of an adaptive response which func-

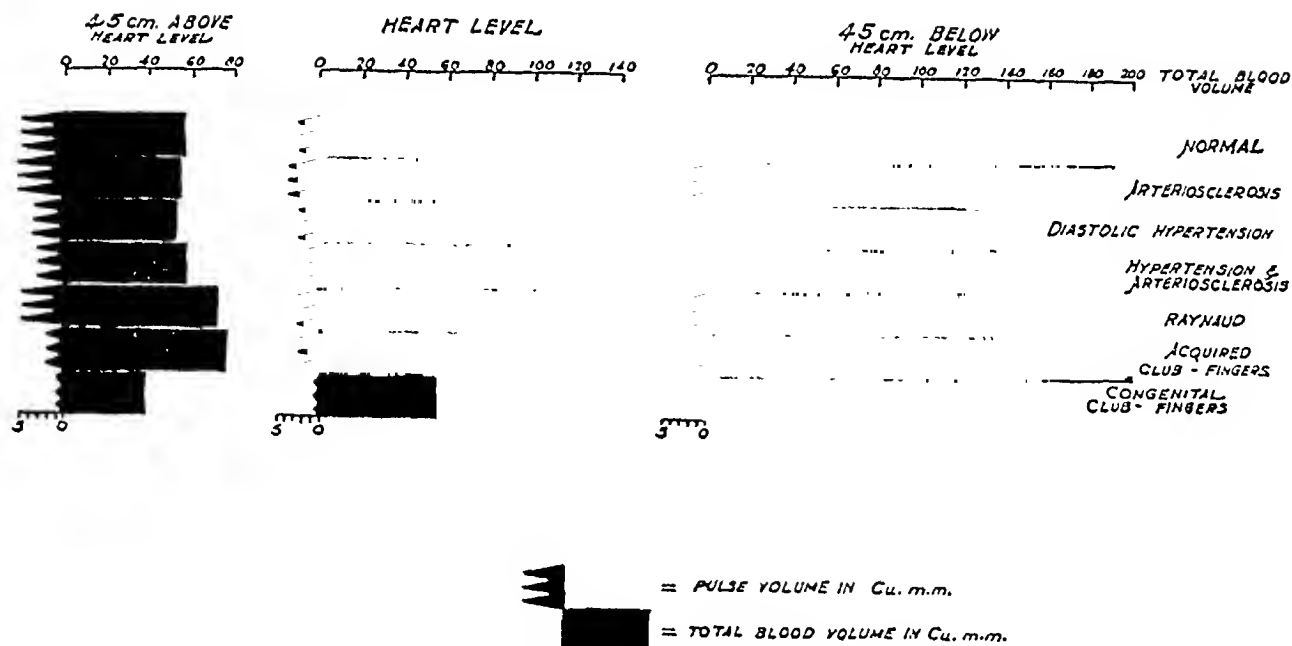


FIG. 1. MEAN VALUES FOR PULSE VOLUME AND TOTAL BLOOD VOLUME OF THE FINGER TIP AT HEART LEVEL AND AT 45 CM. ABOVE AND BELOW HEART LEVEL FOR VARIOUS GROUPS OF SUBJECTS STUDIED

factors involved in the expression of the volume pulse of the finger tip into quantitative values in spite of the fact that sources of error were obvious. We have used pulse pressure as representing the force producing expansion and as being unchanged by position. In our data for normal subjects there was no significant correlation between pulse pressure and pulse volume. Even in one subject, from day to day, pulse pressure and pulse volume varied in such a way as to show that they had no constant relationship. At least one other important variable seemed to be present. The ratio of pulse pressure to pulse volume gives a number which is useful in appreciating the magnitude of influences other than pulse pressure responsible for pulse volume. The volume response to a given pressure change would be modified by the number, size, length and distensibility of the vessels and other factors which would modify friction to blood flow. Changes in both size and tone of vessels most concerned with regulating pulse volume tend to give a larger volume change to a given pressure change, when the change is in the direction of less friction and *vice versa*. According to this mode of analysis, tone and friction are both great when the finger tip expands little in response to a given pulse pressure, and tone and friction are both low when it expands much to the same pressure. The greatest source of error probably lies in the use of pulse

pressure as measured in the brachial artery and not in the arteries entering the finger tip. It seems likely that the pulse pressure in the finger tip arteries is so related to that in the brachial artery as to warrant the use of the latter in preliminary analyses based on this ratio. The factors modifying the transmission of the pressure pulse wave in larger arteries, such as the radial, are the same for the small vessels of the finger tip discussed above. Pulse pressure may diminish, that is the pulse wave may be smoothed as it passes along an artery, by either of two mechanisms involving opposite conditions, great increase in distensibility or a great increase in friction. We have observed (7) in normal arteries changes in pulse wave velocity when the hand was changed from heart level to the depressed and elevated positions indicative of increased tone of the arteries of the arm in the depressed position and diminished tone for the elevated position. Unless these changes were extreme, that is producing marked change in friction, and the changes in pulse wave velocity were not extreme, the influence of changed tone would be toward increased pulse pressure entering the depressed finger tip and diminished pulse pressure entering the elevated finger tip. In small, rigid, calcareous radial arteries the friction factor would probably predominate rather than loss in elasticity, the result

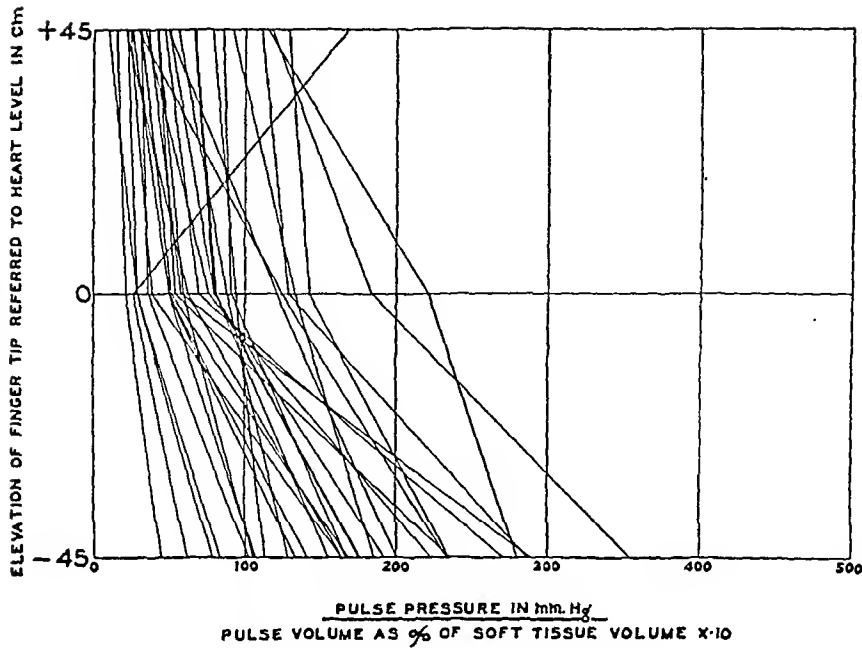


FIG. 2. RATIOS FOR NORMAL ADULT SUBJECTS

Each line connects points which represent a ratio for each of three levels and based on one observation for a normal adult subject. The heavy line represents mean values for ratios.

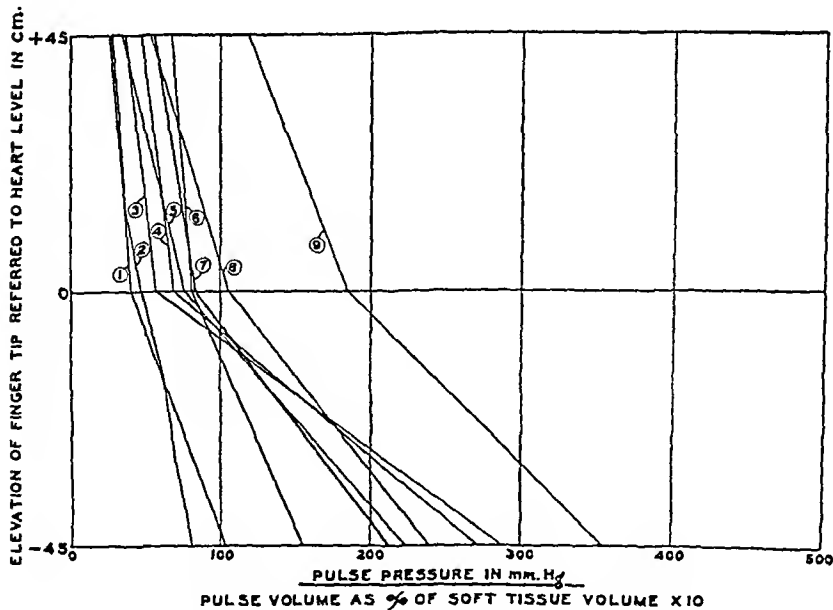


FIG. 3. RATIOS BASED ON REPEATED OBSERVATIONS ON DIFFERENT DAYS FOR THE SAME NORMAL ADULT SUBJECT

Line 2 represents ratios based on 35 measurements of pulse volume at one sitting and seven levels of the finger tip: at heart level and at 15, 30 and 45 cm. above and below heart level. Observations were made nine times at heart level, six times each at the 15 and 30 cm. levels and three times at the extreme positions. Ratio for each level is based on mean of all measurements of pulse volume at that level.

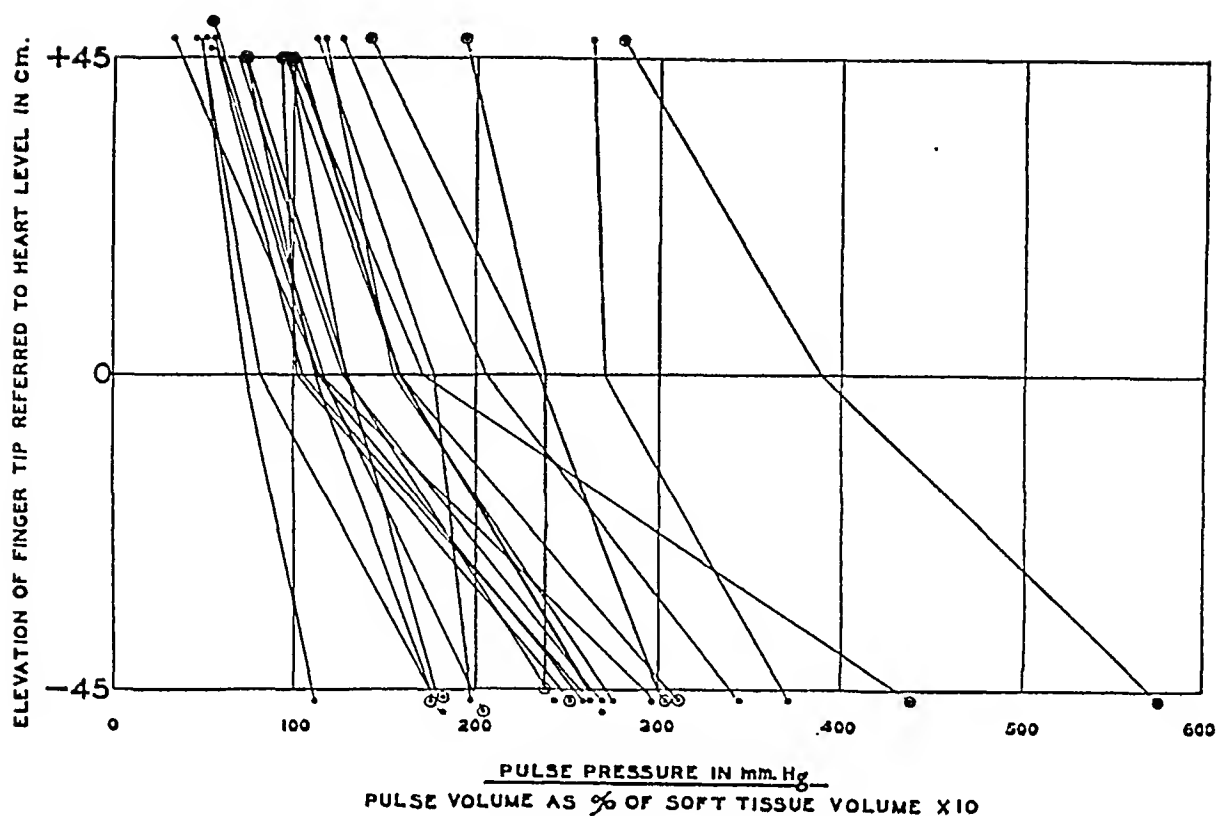


FIG. 4. RATIOS FOR PATIENTS WITH DIASTOLIC HYPERTENSION

Circles represent data for patients who also showed arteriosclerosis. The heavy line indicates mean values.

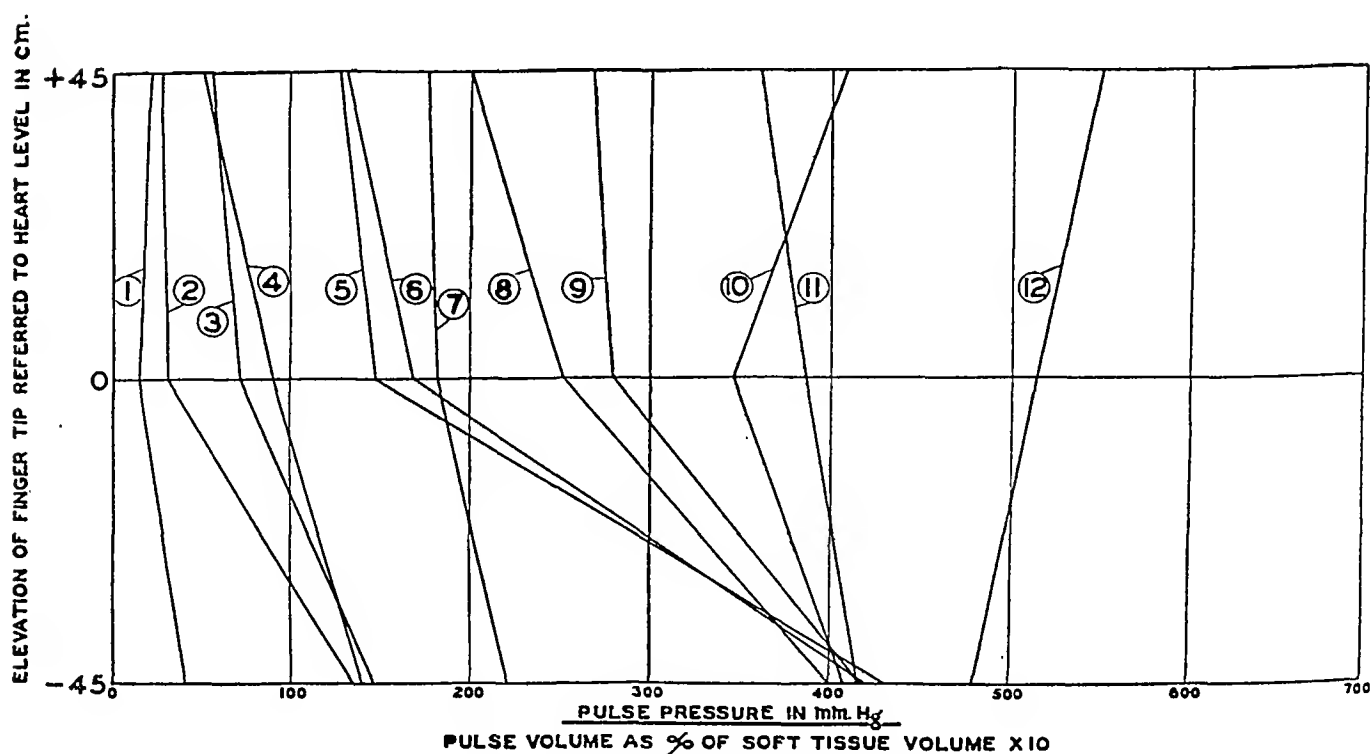


FIG. 5. RATIOS FOR MISCELLANEOUS CASES

(1) acquired clubbing of fingers; (2) arteriosclerosis; (3 and 4) dead finger of Raynaud's phenomenon; (5) arteriosclerosis and mild systolic hypertension; (6) anxiety neurosis; (7) clubbing of fingers of doubtful etiology; (8) extreme arteriosclerosis of arm vessels with atrophy of finger tip; (9) arteriosclerosis; (10) hypertension and congenital clubbing of fingers; (11) extreme arteriosclerosis; (12) congenital clubbing of fingers.

being a greatly diminished pulse pressure in the finger tip arteries.

In the graph in Figure 2 are represented the values for the ratio of pulse pressure to pulse volume for the three levels of the finger tip for the normal adult subjects, and in Figure 3 is shown the analysis of repeated studies made on one normal subject, each on a different day. Scatter is a notable feature which may be due in part to the fact that our subjects were not studied in a basal state, and in part to psychic factors. The graph indicates that tone and friction usually increased more when the change was made from heart level to the depressed position than they diminished when the change was made from heart level to the elevated position. If the graphs had been constructed so that ordinates represented changes in venous pressure, only, this difference would be largely obviated. This would seem to indicate the importance of venous pressure in the adaptive mechanisms with change in position. In the graph in Figure 4 are shown comparable data for patients with hypertension. In these patients the finger tip tended to expand less per unit of pulse pressure than for normal subjects. The calculation of ratios for most of our patients with arteriosclerosis without hypertension gave values within the range of our normal subjects. This is not surprising in view of the irregular distribution of arteriosclerosis. In Figure 5 are plotted ratios of pulse-pressure:pulse-volume for interesting cases of miscellaneous nature. One observation of a normal adolescent subject gave ratios of pulse pressure:pulse volume as high as for any of our patients with vascular disease.

Criteria employed in the selection of subjects with arteriosclerosis were purely clinical. All such patients showed marked thickness of both radial and brachial arteries. Variations in the distribution of the sclerotic process from one portion of the body to another together with the fact that clinical evidence of disease was not in the part studied make it impossible to estimate the extent of the process in the finger tip. Atrophy of the vascular bed which is a part of the pathological process in arteriosclerosis probably explains the reduction in the total blood volume observed in these subjects.

In the group with diastolic hypertension only

those patients with a diastolic pressure of at least 120 mm. of mercury on more than one occasion were studied. According to data shown in Table I, there is a striking difference between the volume increase in the finger tip with depression between normal subjects and patients with diastolic hypertension. This difference has become less marked since observations on additional patients have been included in the analysis. For 40 observations on 33 normal subjects the mean increase in total volume with depression was 111 per cent. For 25 patients with hypertension, 9 with arteriosclerosis also, the mean increase in volume was 71 per cent. These differences are in accord with the views of Fishberg (8) that arteriolar constriction is accompanied by varying degrees of capillary and venular constriction. However, this diminished distensibility of venous vessels may be due to either anatomical or physiological peculiarity, or to both. No differences were noted between the values for total blood volume and pulse volume in patients falling into the groups of red and pale hypertension of Volhard. Volhard considers pale hypertension the result of universal vasoconstriction and red hypertension the result of "diminution of the dilatibility of the large and small arteries resulting from presenile loss of muscular elements with substitution of elastic and collagenous tissue" (8).

In view of the results obtained in subjects with arteriosclerosis alone and diastolic hypertension alone, the fact that subjects with both processes approach more closely those with diastolic hypertension is not surprising.

The results obtained in the subject with Raynaud's phenomenon are explained by the relative reduction in the vascular bed.

The distinctly different results obtained in subjects with congenital clubbing and those with acquired clubbing of the fingers leads one to the possibility that the underlying mechanisms in both types are different, although the duration of the condition may explain the differences. There is no agreement as to the microscopic pathology underlying clubbing of the fingers (9). No bony changes were noted in our cases to explain these findings and we are at present unable to account for them. While our evidence is not sufficient to postulate two distinct types of clubbing of the

fingers, the differences were striking. Further investigations to throw light upon this question are now in progress. Volume of blood in a given tissue at a given moment is of less physiological importance than is the volume of blood which passes through the tissue in a given period of time. Estimations of flow through the finger tip may be made with the same apparatus we have employed in this study by observing the volume change which follows upon sudden venous obstruction. We are at present engaged in making such studies.

#### SUMMARY

Changes in total blood volume and pulse volume of the finger tip due to elevation and depression 45 cm. from heart level for a group of males including normal subjects and patients suffering from various vascular abnormalities are reported and discussed.

Pulse volume increased with elevation and decreased with depression of the finger tip and total volume changed in the opposite direction. The adaptive mechanisms are discussed in terms of behavior of various vessel groups.

The influence of position upon pulse volume is ascribed to change in distensibility of both arterial and venous vessels and to changes in frictional resistance to blood flow and consequent changes in smoothing effect on the pulse wave which are predominantly arterial.

Observations in patients with arteriosclerosis, Raynaud's phenomenon, congenital and acquired clubbing of fingers and one patient with luetic aortic regurgitation are discussed.

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# A CLINICAL STUDY OF THE ACTION OF 10 COMMONLY USED DRUGS ON CARDIAC OUTPUT, WORK AND SIZE; ON RESPIRATION, ON METABOLIC RATE AND ON THE ELECTROCARDIOGRAM

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The possession of a technique which permitted rapid estimations of cardiac output and which, demanding no intelligent cooperation, seemed especially suitable for use on ward patients, has permitted an extensive study on the action of common drugs on the heart and circulation in clinical conditions. This study contains about 450 estimations of cardiac output performed on 85 patients.

Coincidentally with these estimations the action of drugs on pulse rate, on blood pressure, on respiratory rate and volume, and on metabolic rate was observed. Orthodiagrams and electrocardiograms were secured also. Therefore, certain parts of our study dealt with effects already well known.

The results of such estimations have permitted the calculation of heart work, of peripheral resistance, of arteriovenous oxygen difference, and of the ratio of heart work to heart size, the latter a factor of decisive importance in our conception of cardiac stimulation and depression. Therefore our study demonstrates the effect of drugs on these functions also.

Most of the drugs selected are commonly used in cases of cardiac and circulatory disease. We have studied the actions of digitalis, epinephrine, ephedrine, caffeine, theophylline, carbaminoylcholine, sodium nitrite, nitroglycerine, pitressin, quinidine, morphine and strychnine. We have studied the effects of drugs in those clinical conditions in which physicians are accustomed to employ them. But when suitable cases were not available the effects were studied in other conditions.

Almost without exception our results support the general conceptions of drug action derived from animal experiments.

## PROCEDURE

All estimations were performed in the morning. The patients received no food after their evening meal and no water after midnight. They were taken from the ward in bed or in a wheel chair. An electrocardiogram and an orthodiagram were obtained first. Then the subjects lay down for at least 45 minutes. Duplicate estimations of cardiac output and metabolism were then made, together with repeated determinations of pulse rate, blood pressure, respiratory rate and volume.

If the study concerned a rapidly acting drug, this was administered soon after the control estimations. The patient was watched until evidence of the drug's action became manifest objectively. Duplicate estimations of cardiac output and metabolism were then made, the purpose being to make these determinations at the height of action. Orthodiagrams and electrocardiograms were secured immediately afterward.

Cardiac output was estimated by the method of Starr and Gamble (1), the analyses being performed by the katharometer method of Donal, Gamble and Shaw (2). Metabolism was estimated from samples of expired air drawn from a mixing bottle containing a fan.

Respiratory volume was obtained by reading the spirometer at frequent intervals. Respiratory rate was counted repeatedly during the period of observations. It is well known that subjects breathing from a spirometer under 3 mm. H<sub>2</sub>O negative pressure, and through valves, tend to breathe somewhat deeper and more slowly than under normal conditions.

The left ventricular work was calculated as described before (3). The peripheral resistance was calculated from the formula used by Bazett et al. (4).

The volume of the heart was estimated from Kahlstorf's formula (5).

The analyses concerned with estimating cardiac output were performed by Donal, those concerned with metabolism by Joseph, Donal or Eagle. The orthodiagrams were made by Margolies. Starr selected the patients from the wards and decided on the drug and dosage suitable for them. Gamble or Starr administered the drugs and, with Joseph, made clinical observations. The statistical analysis was carried out by Starr and Joseph with the assistance of Dr. H. A. Schroeder.

We are indebted to Dr. L. H. Collins for clinical help in the spring of 1934, to many of the staff and interns of the University Hospital for assistance in securing patients and in adopting the therapy employed to assist this study, and especially to our many patients who so willingly cooperated.

### *Conclusions from results obtained by cardiac output methods*

Before the results can be discussed with profit certain conceptions fundamental to our viewpoint must be set forth. The first concerns our method of drawing conclusions from our results. A second group has to do with basic conceptions of cardiac physiology in relation to which our results will be presented. Our approach to this problem differs from that of certain other workers in this field and this difference must be discussed in detail.

Grollman (6) describes his method as accurate to 10 per cent, i.e. changes of less than this amount might be accounted for by errors inherent in technique and analysis. This conception is supported chiefly by a long series of estimations made on a normal subject who gave results varying within this limit. That changes of less than 10 per cent are not significant is thus rendered probable, but one should not conclude that all changes greater than 10 per cent are significant. To give a patient a drug and to attribute to its action any change of cardiac output of over 10 per cent would be highly hazardous. Duplicate estimations of cardiac output by the acetylene method differ by far more than 10 per cent in many patients. Our method shows similar variations. Standards derived from the best subjects under ideal conditions cannot be used with safety to estimate the significance of differences in clinical work. It is not sufficient to estimate variability due to errors inherent in the method. There is a large variability inherent in subjects, who may change their cardiac outputs due to apprehension and excitement, or induce irregular errors due to poor cooperation. Unless this type of error is considered the conclusions drawn may be highly erroneous.

We have, therefore, employed statistical procedures to estimate the significance of our differences. The method is not a perfect one for our purpose, but it provides the best criterion for the

significance of quantitative data. A detailed discussion of the means employed follows.

In order to estimate the significance of average results obtained on a number of patients given a single drug we have followed exactly the procedure given by Fisher (7, page 104) and made use of his Table IV, to obtain the probabilities. We have defined significance in the customary manner, i.e. a probability larger than 95 in 100 that the result obtained was not due to chance. But we wish to point out that this definition is arbitrary and probably too rigid for clinical work in which physicians constantly are being forced to make important decisions based on data whose probability of error is enormously greater than 5 in 100. Therefore when, after the administration of certain drugs, we have not demonstrated changes of sufficient magnitude and constancy to merit the term significant, we do not imply that these smaller changes should be neglected. They often represent a better picture of the drug's action in clinical conditions than was available before, although not as perfect as one would like. When data become more ample the significance of the smaller changes may be established.

Since no two patients are identical, the question might be raised whether it was proper to draw conclusions from an average of the results obtained. The answer is that we are doing the best we can with the data at our disposal. In the future when series of more similar conditions and dosages are available, more accurate conclusions can be drawn concerning the action of drugs under special circumstances.

Something must be said concerning our ideas of the conclusions which should be drawn from the results obtained from one or two estimations of cardiac output, made on one patient before and after the administration of a drug or other agent. The discussion which follows probably applies to results obtained by Grollman's acetylene method also, for the variation of duplicate estimations found by Nylin (8) on patients by this method agrees closely with that we obtain on patients by our method.

Single estimations, before and after the administration of a drug, give one no knowledge of the spontaneous variability inherent in method and subject, and this makes accurate conclusions im-

possible unless other data on variability are available.

Duplicate estimations, before and after, provide one with a very imperfect measure of the inherent variability, and the data can be handled statistically. They may be considered as two experiments and handled, as we have handled the larger series, according to Fisher (7, Section 24). A very steady subject will have to be used or a very large change found before significance can be attained.

However, long experience with a method permits one to set up other criteria. We have proceeded as follows. A sample was taken consisting of the last 65 pairs of duplicate estimations made on patients in this investigation. The standard deviation computed from the deviation of each of the duplicates about the mean of each pair was found to be 5.6 per cent.

An example will make our procedure clear. Below we calculate a testing standard deviation from two pairs of duplicates in the same manner as was employed for the 65 pairs.

Patients	Duplicate cardiac outputs	Mean of duplicates	Deviation from mean	Deviation from mean of deviations squared
	<i>liters per minute</i>		<i>per cent</i>	
A	3.0, 3.2	3.1	+3.2 -3.2	10.2 10.2
B	4.0, 4.6	4.3	+7.0 -7.0	49.0 49.0
		mean of deviations = 0		Σ118.4
		$\sigma = \sqrt{\frac{118.4}{4}} = 5.4$ per cent		

If one has duplicate estimations before and after the condition inducing change: standard deviation of difference between the means =  $\sqrt{\frac{\sigma^2}{2} + \frac{\sigma^2}{2}}$ .

If one has only one observation before and after: standard deviation of difference between individual estimates =  $\sqrt{\frac{\sigma^2}{1} + \frac{\sigma^2}{1}}$ .

Estimates of the probabilities for any difference may be obtained from Fisher's Table (7, p. 139). In actual practice it is more convenient to think in terms of differences between the results of two estimates, expressed in percentage of their mean, than in the deviations from their means. The first is, of course, twice the second. In the text the conclusions have been expressed in terms of differences.

At the suggestion of Dr. J. H. Austin this standard deviation, 5.6 per cent, was checked by an estimation from the median divided by 0.955,

a figure derived from Pearson (9), which gave an identical value. We propose to use this figure, 5.6 per cent, as a testing standard deviation, a measure of the variability to be expected in the method applied to an average subject.

Employing this figure we find that when we have made one estimation before and one after the agent inducing change, differences between the results of 32, 30, 25, 20 and 10 per cent of their mean have probabilities of 95, 94, 88, 60 and 45 to 100 that they are not due to chance. Differences of over 32 per cent are significant.

If one has results of duplicate estimations before and after the agent studied, differences between the means of each pair of 22.4, 20, 15, 10 and 5 per cent have probabilities of 95, 92, 82, 63 and 45 to 100 that they are not due to chance. In this case differences of over 23 per cent are significant.

Obviously, when estimations are made on the same patient under the same conditions but on different days, the inherent variation is likely to be larger than in duplicate estimations on the same day. In order to make a basis for interpretation of prolonged experiments with drugs we have estimated the variability of the average of duplicate determinations obtained on one day with the same value found on a subsequent day. Discarding the trained subjects and also those patients whose clinical conditions had changed materially between the observations, the needed data were available on 18 patients, and the standard deviation was 7.7 per cent. Therefore, if the difference between the means of duplicate estimations was 30, 20 or 10 per cent the probabilities would be 92, 75, and 44 in 100 that the difference was not due to chance. Differences of over 35 per cent are significant.

This use of a testing standard deviation has disadvantages which should be obvious; for example, some subjects, as cases of hyperthyroidism, are notoriously unstable, undergoing far larger fluctuations in cardiac output than the average subject. To use our testing standard deviation in such a case would be hazardous. Excessive divergence of duplicate estimates would aid in identifying some such cases but there is no way to protect oneself against the occasional error. We are chiefly concerned with recording the exact method by which we draw conclusions

from our data. Then, if improvement in this method occurs to us or to others, correction of our conclusions will be easy. For this reason we have not increased our testing standard deviation by adding multiples of its standard error as is the practice of some statisticians in somewhat similar situations.

### *Cardiac stimulation and depression*

In recent years drugs have been classified as cardiac stimulants or depressants when they increased or diminished contractility of cardiac muscle, as measured by levers attached in animal experiments. Soon after techniques for measurement of cardiac output were available, it became evident that the administration of these drugs was not always followed by a change in cardiac output in the direction which their action on preparations of cardiac muscle had led one to expect. In our opinion, results obtained by the two types of experiments can be reconciled if certain well known facts of circulatory physiology are kept in mind.

Most drugs which affect the heart act elsewhere on the circulation also and changes in the latter may cause secondary changes in cardiac behavior. Before a drug can be spoken of as stimulant or depressant to cardiac function the nature of the secondary changes must be carefully considered.

It seems obvious that the cardiac output is not dependent on the heart alone and that it should not be used as a test of cardiac capacity (10). The normal behavior of the heart, when its inflow and the resistance against which it works are changed, has been described by Starling (11) as follows: the work per beat of the normal heart is related to its size. This "Law of the Heart" also holds for clinical conditions, as closely as the relation between basal metabolic rate and body surface, and more closely than any similar relationship studied (12). From it has been derived a satisfactory definition of cardiac stimulation and depression. A heart is stimulated when its work per beat, in proportion to its diastolic size, increases. It is depressed when the reverse occurs (13). When one applies this definition to the drugs that we have studied the supposed dis-

crepancies between the results obtained on man and in animal experiments largely disappear.

Therefore, we have estimated cardiac stimulation and depression by plotting our results in diagrams (Figures 1 and 2) showing the normal relationship between left ventricular work per beat and heart volume, derived from formula 2, Table V, of a previous paper (12). If the values are changed by the administration of drugs so that the point representing them moves upward or to the left we conclude that stimulation has occurred, for the heart's work in proportion to its size has increased. Movement to the right or down indicates cardiac depression. Movement parallel to the line *AB* defines neither stimulation nor depression but represents the response a normal heart would make to changes elsewhere in the circulation, such as diminished venous return, increased arterial pressure, etc.

Difficulties in the clinical study of cardiac work are obvious. Only the work of the left ventricle can be estimated. There is some evidence from animal experiments that the work of the right ventricle is proportional to that of the left, but this could not be expected to hold in certain clinical conditions. Fortunately, the right ventricle's work is only a fraction of that of the left so that large percentage changes in right ventricular work would make but a small percentage change in the total cardiac work. There being no known method of estimating in the clinic the blood pressure in the pulmonary artery we have ignored the work contributed by the right ventricle in our calculations, although we are fully conscious of the errors which may be involved in certain instances such as mitral stenosis.

Bay (15) has suggested an ingenious method for estimating the amount regurgitated in aortic insufficiency. This gives accurate results on his schema, but it is not intended for use in the clinic. However, he has recalculated the left ventricle's work in three of our published cases and concludes that our estimate is far too small. Bay points out that, before his formula would yield an accurate result, the following conditions would have to obtain: (1) the usual blood flow to the tissues must be maintained, (2) the peripheral resistance must be unaltered, (3) elasticity must be normal. But there is evidence that peripheral resistance is reduced in aortic regurgitation (16),

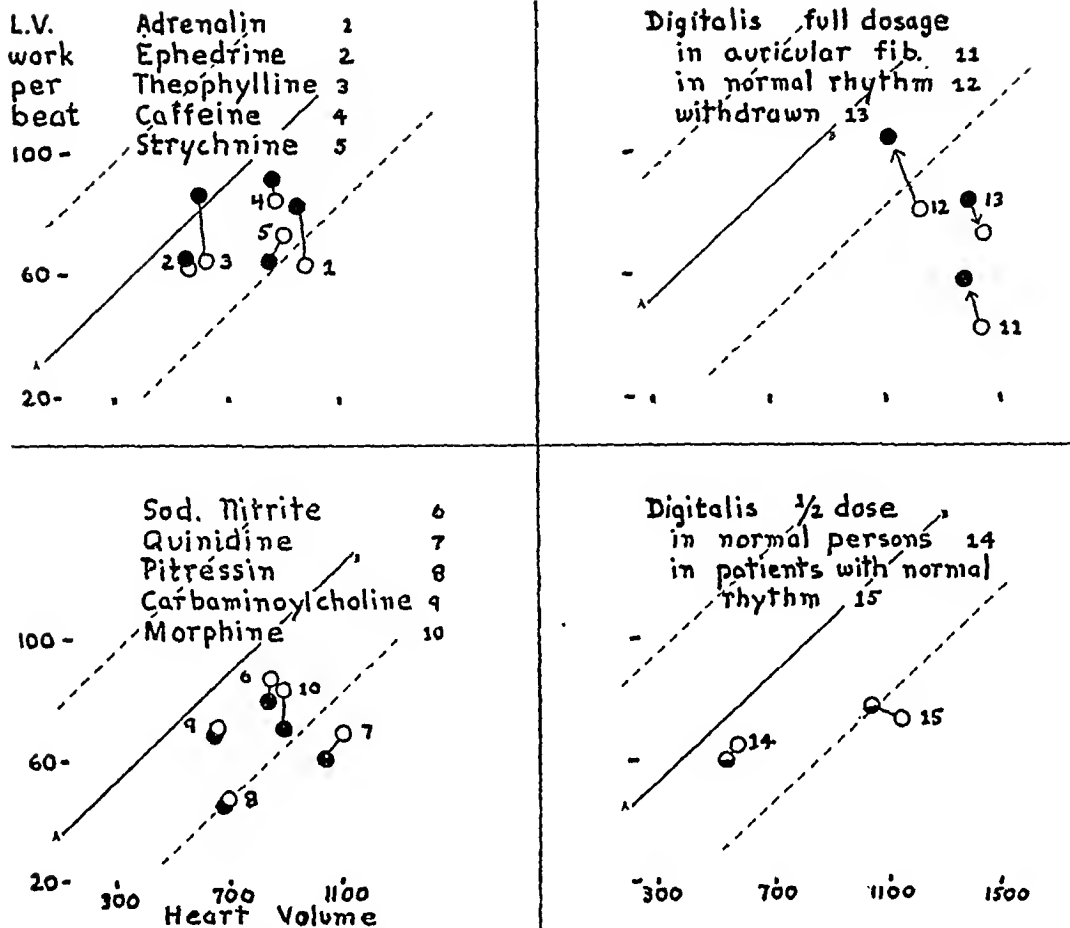


FIG. 1. EVIDENCE OF CARDIAC STIMULATION OR DEPRESSION BY DRUG ACTION—AVERAGE RESULTS

The solid lines *AB* are the calculated best line for persons without heart disease. The outlying dotted lines have been placed at twice the standard deviation from *AB* and define the normal zone, as in Figure 3 (12). According to our definition movement of points at right angles to *AB*, upwards and to the left, indicates cardiac stimulation, the reverse movement depression. The circles indicate the position of average values before drug administration, the solid dots are average values on the same patients during drug action.

Ordinates in grammeters per beat. Abscissae in  $\text{cm}^3$ . The graphs are not absolutely identical with Figures 3, 4 and 5 (12) because a different method of calculating heart volume was used.

that distensibility decreases with age and in disease (17), and that normal blood flow is not always maintained (12). We also question the assumption that blood flow varies as the square root of the pressure applied, a condition well known to occur in hydraulics, and which doubtless holds for Bay's schema, but apparently not for the circulation (18). The expected deviation of most of these items from the basic assumptions of the formula would make the actual aortic leak far smaller than that calculated by Bay. The difficulties involved can be illustrated by pointing out

that the formula as it stands will calculate the amount of "aortic leak" when applied to data from any case with a large pulse pressure, quite irrespective of evidence of the presence of aortic regurgitation. For these reasons we have not changed our method of calculating work in the cases of aortic regurgitation in this series. We gladly admit, as Bay points out, that each result is too small by the amount of the leak and welcome Bay's study as the first attempt to improve the estimation. Complete data have always been given in our tables to permit any one interested

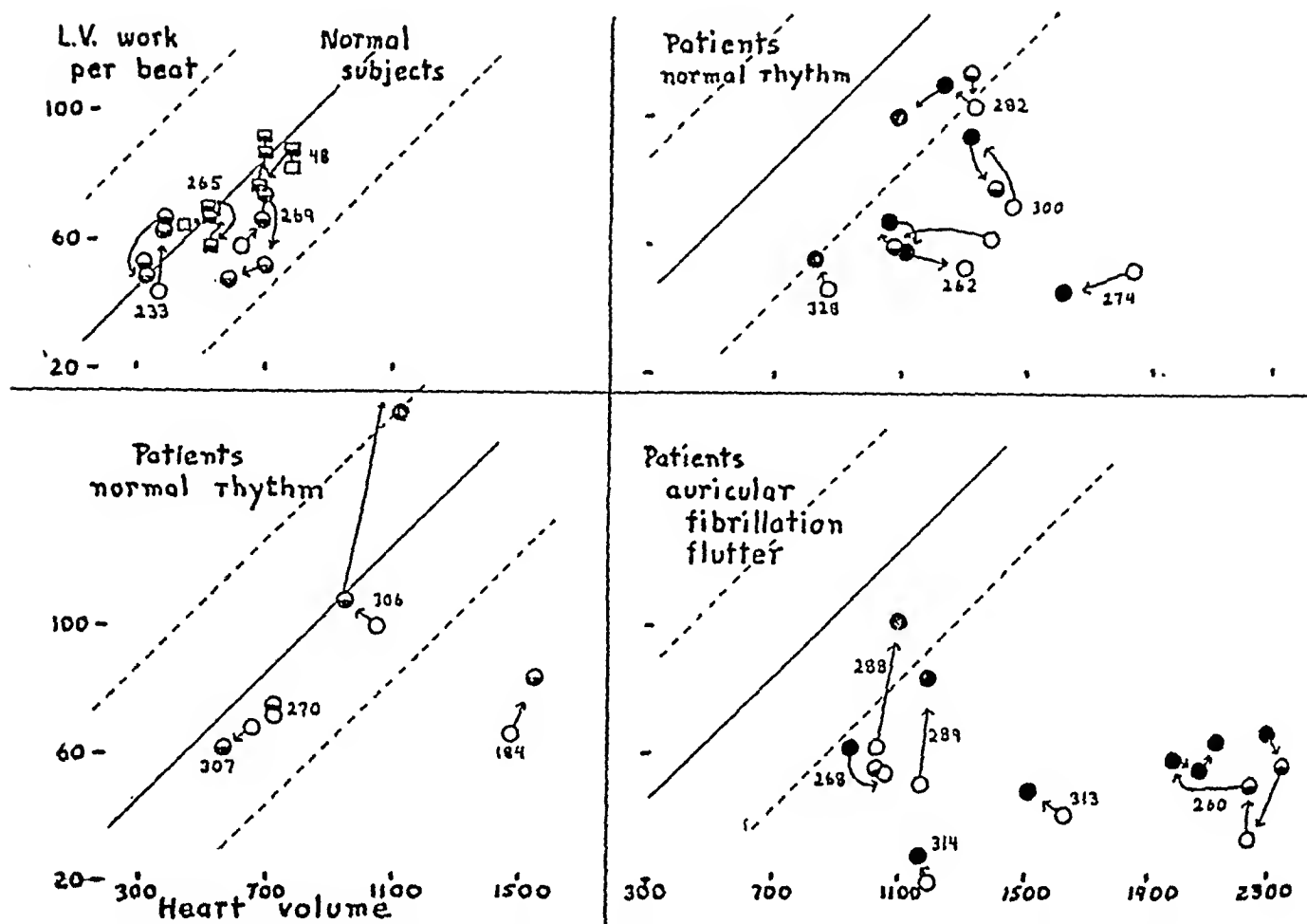


FIG. 2. EVIDENCE OF CARDIAC STIMULATION BY DIGITALIS. RESULTS IN INDIVIDUAL CASES

Coordinates and lines as in Figure 1. Empty symbols indicate values in cases who had received no digitalis for 2 weeks or longer. Dots indicate values obtained during full digitalis action. Symbols half filled indicate the intermediate state, e.g. values obtained after half doses of digitalis, or in patients, previously exhibiting full action, who received no digitalis for one week. Case numbers correspond to data in Table III.

to recalculate the work according to newer and better methods as they become available. Being concerned with changes, the absolute values are of minor importance in this study.

#### RESULTS

The diagnoses, dosages, and other clinical data, together with the results on each case, have been assembled in Tables II and III at the end of the paper. The figures given for metabolic rate are averages of duplicate estimations, those for pulse rate, respiration, and blood pressure are averages of more numerous results.

The results of the statistical analyses have been summarized in Table I also at the end of the paper. The probabilities are given as the next higher figure in Fisher's table, e.g.; 0.209 is recorded as 0.3 etc.

In Figure 3 the results have been presented in a graphic form which has been found useful for teaching medical students.

#### Digitalis

Every patient given this drug had severe cardiac disease; some had recently recovered from congestive failure, others were considered threatened with it, but none had constant râles at the lung bases or the other classic signs of this condition when our tests were made.

As several days must elapse before the effects of digitalis can be secured in the clinic, and about two weeks pass before such effects disappear after discontinuing the drug, the investigator is faced with the problem whether changes detected are due to action of the drug, or to changes in the patient's condition, either spontaneous or induced

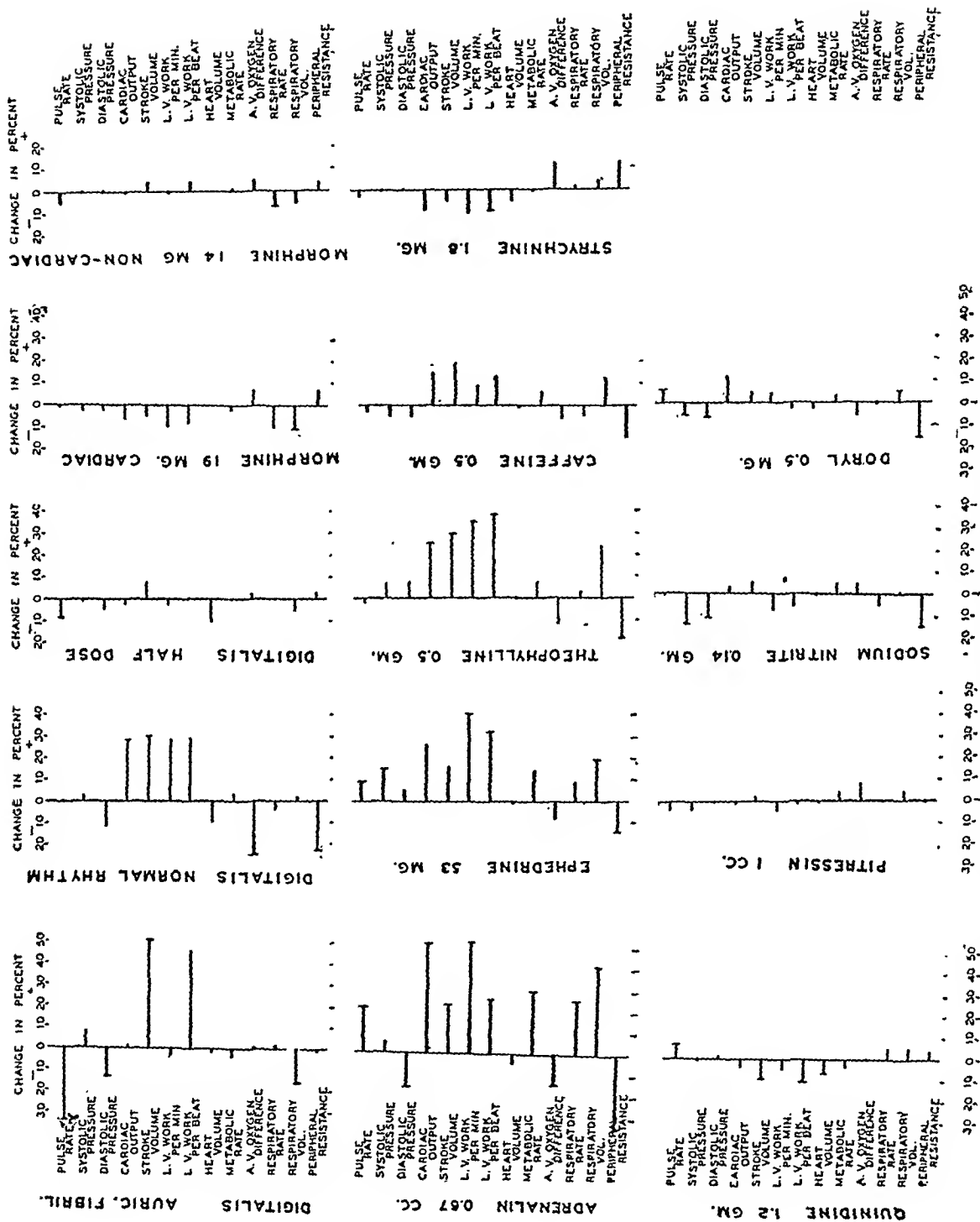


FIG. 3. GRAPHIC PRESENTATION OF THE AVERAGE RESULTS TO PERMIT EASY COMPARISON BETWEEN THE ACTIONS OF THE DRUGS STUDIED. A cross bar at the end of any main bar indicates that the change is statistically significant.

TABLE I  
Statistics on the effect of drugs on heart, circulation, respiration and metabolic rate \*

Drug and remarks	Number of cases	Average dose	Pulse rate	Blood pressure		Cardiac output	Stroke volume	Left ventricular work per minute	Left ventricular work per beat	Heart volume	Metabolic rate	Arterio-venous O <sub>2</sub> difference	Respiration		Peripheral resistance
				Systolic	Diastolic								Rate	Volume	
Digitalis (in auricular fibrillation).....	5	Full	-33.0 0.01	+7.6 0.3	-12.2 0.05	+1.3 1.0	+50.5 0.05	-4.1 0.3	+45.0 0.1	-1.7 0.5	-3.5 0.7	+0.7 1.0	+1.8 0.3	-15.9 0.01	-1.3 1.0
Same, 3 best cases.....	3	Full	-37.1 0.05	+15.2 0.3	-18.5 0.2	+10.3 0.3	+69.2 0.1	+0.9 0.3	+68.6 0.05	-0.4 1.0	+4.9 0.7	+4.0 0.9	+2.9 0.3	-14.1 0.3	-3.3 0.9
Digitalis (in normal rhythm)...	5	Full	+0.2 1.0	+2.8 0.3	-10.1 0.4	+23.2 0.01	+30.0 0.05	+27.9 0.2	+28.1 0.1	-8.0 0.3	+0.3 1.0	-20.5 0.01	-3.3 0.4	+2.1 0.3	-22.9 0.01
Digitalis (in normal rhythm)...	4	One-half	-7.9 0.02	-1.0 0.3	-4.3 0.4	-2.2 0.7	+7.0 0.5	-4.1 0.3	+1.3 0.8	-9.5 0.3	+0.9 0.9	+2.4 0.7	-0.1 1.0	-4.8 0.5	+2.7 0.8
Epinephrine.....	6	0.7 mgm.	+21.0 0.03	+4.3 0.3	-14.5 0.01	+51.7 0.01	+22.7 0.03	+52.4 0.01	+26.2 0.05	-4.0 0.2	+30.4 0.01	-13.3 0.05	+26.0 0.05	+12.0 0.01	-35.1 0.01
Ephedrine.....	6	53 mgm.	+9.7 0.02	+14.8 0.02	+5.2 0.2	+27.0 0.5	+15.7 0.3	+41.2 0.02	+32.7 0.05	-1.0 0.8	+14.2 0.1	-8.8 0.1	+0.2 0.3	+19.4 0.02	-12.9 0.01
Caffeine.....	4	0.5 gram	-2.4 0.6	-4.0 0.1	-3.8 0.1	+14.8 0.3	+19.1 0.4	+9.3 0.5	+13.3 0.4	-0.8 0.5	+6.9 0.7	-6.1 0.6	-4.1 0.4	+12.6 0.6	-14.3 0.3
Theophylline.....	7	0.48 gram	-1.7 0.6	+6.8 0.3	+7.0 0.1	+25.8 0.01	+29.4 0.01	+35.4 0.01	+38.0 0.01	-0.3 0.9	+7.8 0.2	-12.4 0.1	+2.7 0.7	+23.3 0.2	-18.9 0.05
Carbaminoylecholine.....	6	0.5 mgm.	+7.6 0.01	-6.5 0.03	-7.2 0.02	+12.3 0.1	+3.7 0.3	+4.9 0.5	-2.6 0.7	-2.0 0.5	+3.5 0.4	-5.5 0.6	-0.2 1.0	+5.6 0.01	-16.6 0.01
Sodium nitrite.....	7	0.13 gram	-0.8 1.0	-13.3 0.01	-11.5 0.01	+3.4 0.3	+5.0 0.7	-8.5 0.3	-6.3 0.4	-0.1 1.0	+5.0 0.1	+4.9 0.6	-4.6 0.3	+1.0 0.8	-15.4 0.05
Pitressin.....	7	20 units	-3.7 0.1	-3.6 0.3	-0.2 1.0	-1.8 0.9	+2.0 0.3	-4.5 0.6	-1.9 0.9	-1.4 0.6	+3.3 0.5	+8.7 0.3	-0.5 1.0	+3.9 0.6	-1.2 1.0
Quinidine.....	8	1.2 grams	+7.7 0.05	-1.4 0.6	+1.2 0.7	-3.6 0.6	-9.5 0.05	-4.3 0.5	-10.9 0.05	-6.7 0.05	-3.3 0.3	+0.1 1.0	+5.7 0.4	+5.8 0.2	+3.7 0.6
Morphine (cardiacs).....	8	19 mgm.	-1.7 0.6	-2.7 0.4	-2.5 0.1	-7.3 0.2	-5.3 0.4	-9.7 0.1	-8.0 0.3	—	-2.3 0.5	+7.5 0.4	-10.0 0.1	-10.4 0.01	+7.0 0.2
Morphine (non-cardiacs).....	8	14 mgm.	-4.9 0.1	-0.1 1.0	+1.0 0.3	-1.1 0.9	+4.0 0.6	-0.7 1.0	+4.3 0.6	—	+0.4 1	+4.0 0.7	-7.7 0.05	-6.4 0.2	+3.6 0.3
Strychnine.....	7	1.8 mgm.	-2.6 0.4	-0.2 1.0	+0.6 0.2	-9.0 0.1	-5.0 0.3	-10.8 0.1	-9.7 0.05	-6.3 0.2	-1.6 0.8	+11.5 0.1	+1.8 0.8	+3.8 0.7	+11.9 0.1

\* Mean percentage changes induced by drug action in Roman type  
Probabilities of significance of the means, for  $P = 1$ , in italics

In two cases, first given "half," then "full" dosage, the pulse rate first diminished and then returned to its previous level. It is interesting to recall that repeated doses of digitalis, given in acute animal experiments, often cause a primary slowing followed by an increase of heart rate (22).

#### *Effect of atropine on cases receiving digitalis*

In two cardiac cases, Numbers 288 and 306, we estimated the effects of a subcutaneous dose of atropine on patients having an abnormally slow heart rate under digitalis. Case 288, with auricular fibrillation, was given 0.6 mgm. atropine. The pulse rate increased 19 per cent, the heart size diminished slightly and the metabolic rate increased a little. The cardiac output was unchanged (Table II).

Case 306 in normal rhythm received 1.2 mgm. of atropine. A marked increase of pulse rate followed but the cardiac output diminished significantly. Pulse pressure diminished in both cases leaving mean blood pressure essentially unchanged.

Obviously the striking effect of atropine is on the pulse rate, the other changes were not constant.

#### *The effect of coupled beats*

Purely by accident, we obtained, after Case 260 had received a large dose of digitalis, one set of estimations while coupled beats had supplanted normal rhythm. A few minutes later normal rhythm returned and a similar set of observations was secured. Our confidence in the validity of the differences in cardiac output found is en-



hanced by the fact that we always obtained excellent agreement of duplicate estimations in this patient.

During the period of coupling, the pulse rate was but little faster than in the following normal rhythm but the cardiac output was diminished markedly, the metabolic rate was diminished, and the respiratory rate increased. The change in cardiac output during the coupling was of the magnitude which would be present if the normal beats delivered the same amount as before but the extrasystoles contributed almost nothing to the circulation. Apparently this common complication of digitalis action may cause a profound depression of the circulation. It is of interest that the patient insisted he felt as well during the coupling as before or afterward.

#### *Discussion of digitalis action*

That digitalis benefits patients with certain types of heart disease has been known since Withering. The clinical work on this subject has been reviewed recently (24, 25). The mechanism underlying this improvement has been debated (26). Most conspicuous in patients with decompensated fibrillating hearts, the improvement was attributed by Mackenzie to the change in rate of beating. Clinicians have usually believed that the improvement secured in patients with exhausted hearts, beating with regular rhythm, resulted from an increased contraction, and a larger output (24). This belief was supported by the results of Cushny's experiments on mammalian hearts with the cardiometer and the myocardiograph (22).

The discovery that digitalis decreased the output of the heart of normal dogs (20, 26) was therefore unexpected, as was the opinion that digitalis should be regarded as a cardiac sedative (26). Reduction of cardiac output after digitalis was found in normal men by Burwell, Neighbors and Regen (19) and by Stewart and Cohn (20). We find it also.

Studies upon the effect of these drugs on the output of decompensated hearts have been less concordant. Ringer and Altschule (27) and Lauter and Baumann (28) found an increase in output in weakened hearts, while Kininmonth (29) found increases in some subjects and de-

creases in others. Little confidence can be placed in these observations, however, for they were made with methods based on erroneous assumptions. Eppinger, von Papp, and Schwarz (30) and Schwarz and Schimmer (31) using an oxygen method of doubtful accuracy found a decrease of output in cardiac patients with digitalis, while Ewig and Hinsberg (32) with a carbon dioxide method found no change. In contrast, however, Grassmann and Herzog (33) using the acetylene method of Grollman (6) found an increase in most cases when given digitalis. This finding was supported by acute experiments with intravenous injections of strophanthin, in which the cardiac output was deduced by the pulse-wave-velocity and blood pressure method of Broemser and Ranke (34).

Stewart and Cohn (20), employing Grollman's method, with which they had found a decrease in normal cases, discovered an increased output associated with digitalis in patients with signs of heart failure but without pulmonary congestion. They suggested that the drug increased the extent of the contraction in both conditions, and that the accompanying decrease in heart size, which led to a lower output from a normal heart, resulted in a larger output from one which had been dilated initially.

Using a slight modification of the same method, Friedman, Clark, Resnik and Harrison (35) found no such consistent increase in minute volume in decompensated subjects. Administration of digitalis gave an increase in some cases, but no change or a decrease in others. The direction of the change could not be correlated with the presence or absence of clinical improvement. They attributed the divergent results of Stewart and Cohn to the failure of these investigators to secure adequate mixing of the respiratory gases, and considered that in their own work this had been avoided by a slight prolongation of the re-breathing time. If this is the explanation, we are left with the impression that slight differences in technique make such great differences in result that our confidence in the accuracy attained is diminished. The difficulties inherent in the application of the gas methods for estimating cardiac output in patients with varying amounts of pulmonary congestion are obvious. But we ac-

TABLE II \*  
Original data and diagnoses of individual cases receiving digitalis

Case num-ber	Sex	Age	Height	Weight	Date	Digitalis before and between observations	Cardiac output (dupli- cate determi- nations)	Pulse rate	Average blood pressure	Average O <sub>2</sub> consumption	Respiration		Heart vol- ume	Clinical evidences of drug action	Notes on the form of the electrocardiogram		Diagnosis and remarks
											Rate	Vol- ume			P.R.	T waves, etc.	
						grams or cc.	liters per minute	per minute	mm. Hg	cc. per minute	per minute	liters per minute	cc.		sec- onds		
DIGITALIS—NORMAL PERSONS																	
48	M.	40	67	153	Oct. 15, 1934	None	3.8 3.6	55	105-74	231	9	5.1	776	None		Normal	Normal
					16	11.5 cc.	4.6 4.1	59	102-74	245	11	5.2	776	None			
					18	No more	3.4 2.9	53	109-79	232	11	5.3	682	None			
					17	No more	4.1 3.8	55	103-75	227	10	4.9	697	None			
					19	No more	3.8 3.9	53	108-82	230	9	5.4		None			
233	F.	47	62	114	Dec. 3, 1934	None	2.7 2.9	59	99-69	154	11	3.2	366	None		Normal	Normal
					5	8.5 cc.	3.2 3.3	61	105-71	140	12	3.1	385	None		T1 isoelectric, T2 up 0.5 mm	
					5	No more	3.5 3.5	65	108-74	182	13	3.5		None		T1 diphasic, T2 inverted 3 mm	
					6	No more	2.3 2.7	57	101-72	153	8	3.6	330	None			
					7	No more	2.7 2.5	60	106-65	158	11	3.8		None			
285	M.	26	65	144	Nov. 26, 1934	None	3.1 3.5	64	111-76	191	12	4.8	451	None		Normal	Normal
					27	11.0 cc.	3.8 3.4	74	117-86	222	11	5.0	527	None		Unchanged	Normal
					27	No more	4.0 3.1	70	114-84	231	13	5.5		None			
					28	No more	3.1 3.3	71	116-70	211	14	5.3	536	None			
					30	No more	3.9 2.9	65	111-81	196	14	4.5		None			
269	M.	30	68	169	Dec. 10, 1934	None	3.0 3.4	63	108-61	280	9	5.5	629	None		Normal T2 up 2 mm	Normal
					11	12.7 cc.	3.5 3.1	59	109-65	233	12	5.6	688	None		T2 isoelectric	
					11	No more	4.3 4.1	67	110-63	262	11	5.6		None			
					12	No more	2.7 2.9	63	115-57	253	12	5.3		None			
					14	No more	2.6 2.4	60	107-62	290	13	6.5	585	None		T2 isoelectric	
DIGITALIS—PATIENTS WITH ATRIAL FIBRILLATION																	
280	M.	27	69	165	Oct. 5, 1934	Full	2.8 3.1	66	141-87	277	21	6.8	2290	Yes		T2 slight diphasic	Rheumatic heart disease, mitral stenosis, formerly decompensation, Class III
					12	No more	2.4 2.6	68	132-87	280	30	9.5	2344	Yes		No change	At 5 p.m. after carbohydrate lunch
					22	No more	1.6 1.9	79	124-98	237	26	9.1	2230	Slight		No change	Nauseated later in day
					23	1.1 grams	2.6 2.4	72	140-71	253	29	9.3	1990	Yes		T1 deepened	
					25	0.5 gram	1.9 1.9	49	142-80	220	7	9.7		Yes			
					29	No more	1.9 1.9	51	144-70	223	31	10.4	2070	Yes		No change	
					Nov. 2, 1934	No more	2.8	60	140-64	257	16	7.7	2107	Yes			
					2	No more	1.9	68	132-60	227	28	7.1		Yes			
					2	No more	2.8	50	130-70	251	20	7.3	1996	Yes		No change	Coupled beats
268	M.	50	61	124	Nov. 30, 1934	Full	3.5 3.3	81	147-71	283	25	4.5	944	Yes		T 1, 2, 3 isoelectric	Diffuse toxic goiter, Class II B
					Dec. 11, 1934	No more	3.3 3.4	87	131-78	285	19	7.3	1007	Slight		T2 slightly higher	
					14	1.8 grams	2.7 2.6	66	131-69	223	18	7.0	1060	Yes		T2 isoelectric	
288	M.	75	65	136	Mar. 19, 1935	None	3.2 3.4	108	176-120	237	10	7.9	1032	None		Occasional ventricular extrasystoles	Arteriosclerotic heart disease decompensation 6 months ago.
					22	1.8 grams	3.0 3.0	63	227-90	201	14	6.3	1095	Yes		More ventricular extrasystoles	Class II B
					22	No more	3.0 3.0	75	215-105	235	12	6.9	1025	None		T1, 2, 3 isoelectric	After 0.6 mgm. atropino
313	M.	38	69	141	Nov. 22, 1935	0.9 gram	2.8 3.0	97	127-70	203	18	8.0	1630	None		T1, 2, 3 isoelectric	Rheumatic heart disease, mitral stenosis, recently decompensation, Class II B
					Dec. 13, 1935	3.5 grams	2.6 3.0	68	119-70	262	17	7.0	1520	Yes		No change	
314	M.	58	63	116	Nov. 22, 1935	None	2.0	130	100-80	178	17	7.0	1200	No		T2 isoelectric	Rheumatic heart disease, mitral stenosis, formerly decompensation, Class II B
					Dec. 3, 1935	1.9 grams	1.6 1.6	69	102-75	216	18	6.5	1178	Yes		T2 inverted 2 mm.	

DIGITALIS—PATIENTS WITH AURICULAR FLUTTER

	56	61	160	Apr. 26, 1936 May 1, 1936	None 2.6 grams	2.3 1.0 2.7 2.7	72 155-101 60 177-103	216 234	11 18	6.3 1171 0.0 1210	No Yes	2 to 1, 3 to 1 or 4 to 1 Mostly 4 to 1 flutter	Auricular flutter since 1012
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DIGITALIS—PATIENTS WITH NORMAL RHYTHM, FULL DOSE

	52	07	147	Oct. 30, 1934 Nov. 1, 1934 13	None 1.1 grams 1.2 grams No more	2.0 3.1 2.8 2.5 3.4 3.3 2.7 2.4 2.4 2.0 2.4 2.0	70 134-101 73 133-100 77 126-100 60 130-70 70 120-88	227 224 227 224 242	17 18 17 10 10	8.2 1360 8.4 1067 0.1 1055 8.0 1100 8.1 1280	None None Yes Yes Yes	T2 up 2 mm. T2 inverted 1 mm. T2 inverted 2 mm. T2 diphasic T2 diphasic	Rheumatic heart disease? Pulmonary valvulitis. Former paroxysmal cardiac dyspnea. Class II B Occasional nodal extrasystole Occasional dropped beat Hypertensive heart disease formerly decompensated. On digitalis 0.2 gram for 6 days. Class II B
262 M.	46	63	181	Feb. 19, 1935	0.5 gram daily	3.0 4.1	50 152-93	230	10	10.0 1303	Some	T2 diphasic	
				Mar. 1, 1935	No more	3.0 3.4	60 150-100	249	11	7.2 1315	None	T2 same	
				Mar. 8	2.6 grams	4.8	70 160-98	280	10	0.0 1217	Toxic	T2 upright	
				5	No more	3.4 3.0	60 170-100	208	11	0.3 1070	Yes	T2 diphasic	
300 M.	53	66	160	Oct. 1, 1935	None	3.1 2.7	78 205-163	252	20	7.6 1156	None	T2 slightly diphasic	
				4	2.1 grams	3.0 3.8	63 203-158	246	20	7.0 1308	None	T2 depressed	
				11	No more	3.2 3.2	61 207-151	254	17	7.6 1410	Slight	T2 less depressed	
306 M.	33	67	141	Oct. 22, 1935	None	2.0 3.1	42 124-89	217	16	0.0 1050	None	T2 upright 2.5 mm.	
				25	1.1 grams	2.0 3.0	40 130-93	109	13	5.4 058	Yes	T2 same	
				Nov. 1, 1935	No more	4.5 4.7	43 105-95	218	12	5.1 1030	Yes	T2 higher	
				8	1.3 grams	3.3 3.0	71 151-100	231	15	0.0 1000	Yes	T2 upright 2.5 mm.	
						4.3	45 161-100	205	15	5.3 1120	Yes	T2 lower	
328 M.	41	71	160	Feb. 4, 1936	None	2.0 2.8	105 173-75	351	27	12.3 873	None	T2 upright 3 mm.	
				18	2.6 grams	3.2 4.2	87 130-55	353	25	10.0 810	Yes	T2 upright 2 mm. ventricular extrasystoles	
374 M.	54	64	163	Jan. 15, 1935	None	3.8 3.3	87 120-71	300	10	7.4 1520	None	T2 lower still up	
				25	2.1 grams	2.7 2.8	68 112-57	260	17	0.7 1261	Doubtful	T2 higher	
				Feb. 0, 1935	No more	3.4 3.2	08 116-50	230	18	6.3 1557	None		

DIGITALIS—NORMAL RHYTHM, SMALL DOSE

	72	60	139	Dec. 17, 1934 21	None 0.5 gram	2.5 2.8 2.5 2.5	50 158-55 40 150-55	216 178	13 12	0.7 718 0.8 720	None None	No change	Arteriosclerotic heart disease; slight aortic regurgitation. Class II A
307 M.	22	63	146	Oct. 25, 1935	None	4.2 3.1	78 127-80	294	15	5.0 663	None	Normal	Hypothyroidism, Class II A
				29	1.1 grams	4.2 3.1	73 100-77	320	14	0.4 578	Slight	T3 inverted	
353 M.	48	61	164	Apr. 25, 1935	None	5.4 5.0	85 182-72	281	15	7.2 1080	None		Arteriosclerotic heart disease; slight aortic regurgitation. Outpatient Class II A
				29	1.0 gram	4.1 3.5	73 180-71	200	10	5.4 1045	None		
384 M.	23	73	163	Apr. 25, 1935 May 2, 1935	None 1.5 grams	4.2 3.7 4.5 4.2	74 122-57 64 120-52	314 310	16 17	8.0 1170 6.9 1652	None ?		Rheumatic heart disease; slight aortic regurgitation. Out- patient Class II A

• While any discussion of the value of drugs is outside the scope of this paper, some mention must be made of the beneficial effects of digitalis in our cases as judged by the author in charge of the ward. Of the cases of auricular fibrillation Case 260 was benefited little if at all, and withdrawal of the drug caused little detectable clinical change. Case 262 with hyperthyroidism was not benefited. Cases 288 and 314 were greatly, and Case 313 slightly, benefited. Case 289, the case of flutter, had indefinite cardiac pain after digitalis and it was later abandoned.

Of the patients with normal rhythm Case 262 was much improved and the gain was lost when the drug was omitted. Case 328 was much improved also. In Case 306 the rapid and striking improvement was probably not to be attributed to medication. Cases 274 and 282 were no better after the drug. The cases receiving small dosage showed no improvement certainly due to the drug.

Therefore we do not find any single feature of digitalis action which is invariably correlated with clinical improvement.

cept these results as the best that can be obtained at present under these conditions.

Therefore one might judge from the literature that digitalis exhibited varying cardiac effects in animal preparations, in normal persons, in patients with heart disease and normal rhythm, and in auricular fibrillation. Our results, viewed superficially, would reveal the same thing, for considering all our subjects as one group, the estimated cardiac output after digitalis is diminished in four, increased in eight, varied in four, and was essentially unchanged in four. A similar diversity of results was found by Harrison and his coworkers in congestive heart failure (21).

But by the application of the conception described on page 802 our results can be unified. Utilizing our definition of stimulation and depression, and plotting the average digitalis effects in Figure 1 and the satisfactory individual cases in Figure 2, we find that digitalis stimulates the heart in every instance, i.e. the heart's work per beat, in proportion to its size, increases. Withdraw the drug and the reverse usually occurs. Those cases in which cardiac output diminished after digitalis also have had a diminution of pulse rate, or of heart size, or of both.

We, therefore, support the classic view that digitalis is a cardiac stimulant, a conclusion drawn many times from the results of clinical observation and animal experiment, and we believe that the apparent divergencies of digitalis action can be unified by this conception.

### *Epinephrine*

The second group of estimations was made from 20 to 40 minutes after the subcutaneous injection when tremor of extended hands, and changes in either pulse rate or blood pressure were present. The results, Table III, are similar in most respects to those of our predecessors in this field. Somewhat to our surprise the average mean blood pressure failed to rise after adrenalin in our subjects. The systolic pressure rose in 4 of the 6 subjects but the diastolic pressure fell in every instance. The latter is the usual finding (36) so the effect on mean blood pressure is often small.

Our results (Figure 3 and Table III) show a

significant increase in pulse rate, metabolic rate, cardiac output, cardiac work, and respiration after the drug. There was also a great diminution of peripheral resistance. Marked stimulation of the heart muscle is indicated by the data illustrated in Figure 1.

The electrocardiograms showed no noteworthy change except in Case 299, who, showing only occasional ventricular extrasystoles before the drug, developed short periods of bigeminal rhythm, the extrasystoles replacing every second beat.

The usual effects of epinephrine increasing metabolic rate, systolic blood pressure and pulse rate are well known. Euler and Liljestrand (37) first demonstrated effect on cardiac output on two normal subjects. Field and Bock obtained a similar result (38). Lauber and Brauch (39) secured evidence of a similar effect on 3 patients at the height of action, after a small dose, as 0.01 mgm., given intravenously. The latter authors employed Broemser and Ranke's method (34) in which cardiac output is deduced from the blood pressure and pulse wave velocity. This method has been criticized (4), and we have had no experience which permits us to evaluate its results.

It should be emphasized that the effects of epinephrine on heart and circulation far overbalanced the blood pressure effects on our subjects. Indeed, despite the blanching around the site of injection seen in all our subjects, and the facial pallor seen in some, the peripheral resistance markedly diminished in every case, and one is forced to conclude that vessels dilated at some location. Clough deduced this from the fall of diastolic pressure (40) and Grollman points out the same implication in Euler and Liljestrand's data (6).

It seems reasonable to suppose that local vasoconstriction at the site of injection, due to the high concentration of drug at that spot, so impedes absorption that the minute amounts of drug reaching the general circulation dilate vessels as do extremely minute doses injected intravenously in animal experiments. Reflex vasodilatation from increased pressure due to the cardiac stimulation may be a factor also.

### *Ephedrine*

Changes in blood pressure allowed us to identify clearly the height of action in most instances

TABLE III  
Original data and diagnoses of individual cases

Case number	Sex	Age	Height	Weight	Cardiac output (duplicate determination)		Pulse rate	Average blood pressure	Average O <sub>2</sub> consumption	Respiration		Heart volume	Dose	Diagnosis and remarks
					liters per minute	per minute				Rate	Volume			
		years	inches	pounds			mm. Hg	cc. per minute	per minute	liters per minute	cc.			
EPINEPHRINE														
1	M.	62	73	168	3.3	56	168-75	282		8.1		mgm.		Diabetes mellitus, peripheral vascular disease
					4.3	62	155-58	308		9.6		0.5		Arteriosclerosis
274	M.	54	64	163	3.4	3.2	68	116-59	239	18	6.4	1587		Multiple hereditary telangiectases. Congestive failure 6 months before
					5.3	4.2	67	130-53	312	18	8.8	1425	0.45	Neurocirculatory asthenia
276	M.	47	71	165	3.3	3.1	78	114-65	213	9	5.3	564		
					6.1	5.4	104	112-57	310	14	8.9	554	0.8	
299	M.	52	72	162	4.0	3.7	92	120-105	379	20	11.8	1157		Hyperthyroidism
					5.6	5.5	110	129-84	543	27	16.5	1150	0.5	
326	M.	50	62	156	3.4	3.6	84	136-87	249	11	5.4	815		
					4.5	5.7	82	155-83	304	15	8.0		1.0	a pectoris
333	M.	52	65	152	3.0	4.1	57	118-80	176	12	4.3	690		
					6.3	6.0	79	128-65	230	14	5.9	666	0.75	
EPHEDRINE														
233	F.	47	62	114	2.7	2.9	59	99-70	154	11	3.2	366		Normal
					3.7	3.5	67	115-73	158	14	3.7		50	
258	M.	50	67	130	3.4	3.1	62	96-79	121	17	3.5			(?) Myxedema
					5.1	4.6	68	128-89	177	17	3.8		50	
263	M.	43	68	140	4.1	3.2	58	102-79	223	7	5.1	509		(?) Neurasthenia
					3.4	3.9	61	112-79	232	9	5.8	468	50	
265	M.	26	65	144	3.1	3.5	64	111-76	191	12	4.8	451		Normal
					3.0	4.5	72	118-77	209	15	5.4	456	50	
269	M.	30	68	169	3.0	3.4	63	108-61	280	9	5.5	629		Normal
					4.9	4.5	63	115-70	322	7	7.9		50	
332	M.	62	65	189	2.0	3.0	70	116-69	264	22	7.2	750		Gastro-intestinal influenza
					2.8	3.2	83	135-68	287	23	8.8	781	67.5	Convalescent
CAFFEINE														
281	M.	23	66	145	4.3	3.9	73	138-49	259	10	5.1	920		Post-encephalitis. Rheumatic heart disease. Aortic regurgita-
					6.0	5.6	65	129-46	262	10	5.4	894	.5	(?) mitral valvulitis
282	M.	46	63	181	3.6	4.1	59	152-98	230	16	10.0	1303		Just out of 2d decompensation.
					3.6	4.1	63	145-91	235	14	9.4	1326	.5	
283	F.	32	64	157	3.5	4.2	93	117-75	168	9	4.1	545		neurocirculatory asthenia
					5.5	4.3	92	115-74	239	9	7.1	534	.5	
284	M.	37	70	191	5.3	4.5	67	125-77	268	14	7.5	772		Psychoneurosis. Atypical constant precordial pain
					5.1	3.9	63	123-76	220	14	5.8	772	.5	
THEOPHYLLINE ETHYLENEDIAMINE														
303	M.	16	68	129	2.9	3.2	109	123-75	247	23	7.1	373		(?) rheumatic fever. No known cardiac involvement
					4.8	3.9	100	118-82	245	22	7.5	412	0.48	
306	M.	33	67	141	4.9	4.3	45	155-108	197	15	5.3	1120		Paroxysm of auricular fibril-
					4.9	6.2	50	171-118	253	20	10.3	1055	0.48	fully digitalized
307	M.	22	68	146	4.2	3.1	73	109-77	326	14	6.4	578		calculated dose digitalis
					4.9	3.7	78	105-77	291	13	6.0	550	0.48	
308	M.	37	68	129	3.2	2.6	85	100-74	211	19	5.8	608		Angina pectoris. Xanthomatosis
					3.0	3.3	76	97-75	243	18	6.6	614	0.48	
309	M.	50	70	136	2.5	2.6	56	85-62	201	10	4.5	571		Low backstrain. Possibly neurocirculatory asthenia.
					2.6	2.8	62	107-75	219	10	5.4	565	0.48	
317	M.	47	67	159	3.1	2.3	91	148-117	224	17	5.8	680		Angina pectoris
					3.4	3.5	89	158-130	238	16	6.3	650	0.48	
329	M.	57	66	116	2.0	1.5	54	117-67	178	17	5.1	528		Angina pectoris
					2.1	3.3	53	137-66	201	17	6.0	512	0.48	(?) Duodenal ulcer
CARBAMINOYLCHOLINE														
271	M.	39	63	114	2.9	3.4	73	103-69	226	7	5.5	579		Thromboangitis obliterans
					3.9	3.8	83	87-61	227	7	6.0	555	0.5	
277	M.	44	68	153	3.0	2.9	63	223-127	214	15	5.9	558		Arteriolar nephrosclerosis
					3.0	3.0	71	205-122	235	15	5.0	612	0.4	
315	M.	27	60	166	3.4	3.1	78	125-90	255	17	7.1	755		Headache—no cause found
					4.3	3.3	80	112-86	312	18	7.5	748	0.6	
323	M.	36	60	169	3.0	2.9	52	94-65	230	15	5.8	659		Thromboangitis obliterans
					3.1	3.3	58	93-64	254	15	6.0	656	0.6	
324	M.	30	74	182	4.3	5.0	73	113-71	308	15	8.4	667		Thromboangitis obliterans
					3.7	4.0	75	113-61	313	14	8.9	621	0.6	
327	M.	60	66	131	2.1	2.5	64	181-87	232	14	5.2	720		Arteriosclerotic peripheral vascular disease
					3.2	2.9	68	174-81	208	14	5.5	650	0.4	

TABLE III—Continued

Case number	Sex	Age	Height	Weight	Cardiac output (duplicate determinations)		Pulse rate	Average blood pressure	Average O <sub>2</sub> consumption	Respiration		Heart volume	Dose	Diagnosis and remarks
										Rate	Volume			
		years	inches	pounds	liters per minute		per minute	mm. Hg	cc. per minute	per minute	liters per minute	cc.		
SODIUM NITRITE														
280	M.	32	71	118	8.3 7.5	71	211-135	217	5	6.2	602	grams		Advanced chronic glomerulonephritis
317	M.	47	67	150	5.1 4.7	68	161-112	200	6	9.3	580	0.12		Very nervous. Excited during 1st run
					2.4 3.0	88	137-107	202	17	5.7	660			Angina pectoris
318	M.	35	63	136	3.5 2.5	95	113-92	226	17	5.9	635	0.12		
					4.2 3.7	72	223-167	201	15	5.7	672			Essential hypertension. Former hemiplegia
319	M.	43	66	157	3.0 3.8	73	185-130	195	13	5.4	633	0.12		
					2.2 2.1	61	187-153	215	15	5.2	912			Essential hypertension. Former hemiplegia
					3.5 3.2	59	145-118	221	13	5.1	850	0.12		
320	M.	56	68	157	2.9 2.8	83	231-127	265	22	7.7	800			Renal arteriosclerosis with beginning renal failure
					2.5 2.8	80	217-120	279	23	7.8	836	0.12		
321	M.	55	65	141	2.8 2.6	60	166-115	255	13	6.4	1090			Renal arteriosclerosis with beginning renal failure. Blood urea
					3.3 3.0	61	161-107	276	14	6.2	1055	0.12		nitrogen 30
322	F.	45	64	124	4.6 4.1	122	259-150	268	19	6.1	856			Advanced chronic glomerulonephritis
					3.9 3.0	112	252-153	262	17	6.0	872	0.18		Blood urea nitrogen normal. Died 3 weeks later
330	M.	72	67	193	3.2 2.3	61	141-89	236	14	5.8	955			Arteriosclerotic heart disease. Aortic stenosis?
					2.8 2.5	62	115-85	262	15	6.8	1080	0.12		
NITROGLYCERINE														
1	M.	62	73	168	1.9	51	145-65	221		6.9		mgm.		Diabetes mellitus. Peripheral vascular disease
					3.9 3.1	71	114-98	227		12.1		1.2		Arteriosclerosis
2	M.	63	73	170	1.7	78	195-95	195		6.4				Essential hypertension. Left hemiplegia
					2.2 3.9	05	160-97	275		7.3		1.2		
PITRESSIN														
266	F.	38	65	104	4.3 3.3	70	88-68	163	11	4.0	366	units		Diabetes insipidus
					2.7 2.2	71	89-61	153	10	3.1	331	30		
266					2.0 2.7	64	87-65	139	9	3.0				5 days after 1st test
					2.2 2.7	68	83-61	144	9	3.3		30		
272	M.	23	70	120	2.2 2.5	74	101-62	181	17	5.1	423			Neurocirculatory asthenia
					2.4 2.5	73	94-61	186	24	5.8	377	8		
272					2.3 2.1	70	94-56	191	22	5.8	423			3 days after 1st test
					2.1 2.0	05	86-58	196	19	5.5	405	16		
274	M.	54	94	163	2.7 2.8	68	112-57	260	17	6.7	1604			Multiple hereditary telangiectases. Fully digitalized
					3.0 2.4	67	115-60	288	18	7.1	1565	10		
312	M.	36	63	137	4.3 4.2	65	94-70	220	11	10.8	548			Diabetes insipidus
					4.4 4.6	62	79-57	263	9	13.3	570	10		
328	M.	41	71	160	3.2 4.1	87	139-55	353	25	10.0	819			Syphilitic heart disease. Aortic regurgitation fully digitalized
					2.8 3.1	80	144-66	293	24	9.6	837	10		
293	M.	23	67	132	2.3 2.5	72	103-64	211	14	4.3	498			Diabetes insipidus
					3.5 3.2	70	103-64	235	13	5.5	514	7		Suffed pituitary powder up nose
QUINIDINE														
260	M.	27	69	165	2.8 3.0	66	141-87	277	21	6.8	2290	grams		Rheumatic heart disease. Advanced valvulitis. Auricular fi-
					2.4 2.6	71	141-95	269	30	8.0	2280	1.0		brillation. Formerly decompensated. Fully digitalized
272	M.	23	70	120	2.5 2.0	79	99-52	193	21	5.5	469			Neurocirculatory asthenia
					2.8 2.8	93	102-57	199	22	6.2	424	1.0		
274	M.	54	64	163	3.7 3.3	87	120-71	306	19	7.4	1831			Multiple hereditary telangiectases. Decompensated 5 months
					3.9 3.6	88	107-64	309	20	8.0	1640	1.0		before. No digitalis
275	F.	38	60	112	2.2 2.3	61	137-96	145	12	3.4	715			Rheumatic heart disease; mitral stenosis; auricular fibrillation
					2.0 1.8	55	137-97	134	14	3.2	661	0.6		digitalized
282	M.	46	63	184	3.6 2.4	60	160-100	249	11	7.2	1315			Hypertensive heart disease. Formerly twice decompensated.
					3.1 3.5	70	166-107	244	9	7.0	1336	2.0		No digitalis
282					3.4 3.9	69	176-100	268	11	6.3	1070			7 days after previous test. No digitalis
					3.5 3.5	72	141-90	232	11	6.6	1045	2.0		Improved clinically
285	F.	32	60	127	3.8 3.9	86	196-133	182	16	4.4	551			Arteriolar nephrosclerosis. Nausea and vomiting soon after
					4.5 3.3	95	200-137	189	16	4.8	460	1.4		post-drug run
287	F.	34	63	110	4.1 4.1	97	110-70	187	18	4.9	454			Neurocirculatory asthenia
					3.6 3.0	110	114-69	183	17	5.1	396	0.4		

TABLE III—Continued

Case number	Sex	Age	Height	Weight	Cardiac output (duplicate determinations)	Pulse rate	Average blood pressure	Average O <sub>2</sub> consumption	Respiration		Heart volume	Dose	Diagnosis and remarks
									Rate	Volume			
		years	inches	pounds	liters per minute	per minute	mm. Hg	cc. per minute	per minute	liters per minute	cc.		
MORPHINE (NON-CARDIAC CASES)													
239	M.	17	67	115	5.2 4.4	94	124-80	241	20	6.5			Typhoid fever convalescent
240	M.	58	66	139	3.7 3.3	81	121-76	235	18	5.2		10	Dundentitis
241	M.	24	64	121	3.5 3.8	83	100-92	136	14	3.7	567	15	Arthritis, colitis
4-3-34	M.	24	64	121	3.2 3.2	80	98-84	160	13	4.3		15	Arthritis, colitis
241	M.	24	64	121	3.7 3.5	83	106-78	204	17	5.2	498	15	Arthritis, colitis
4-10-31	M.	24	64	121	4.7 4.4	78	105-84	222	17	4.9		15	Arthritis, colitis
242	M.	29	72	163	3.8 4.4	72	99-77	228	17	4.9	498	15	Chronic colitis
243	M.	35	68	131	3.1 3.8	75	107-84	248	15	4.7		15	Gastric neurosis
244	F.	35	63	117	4.7 5.1	82	109-92	270	11	5.5	514	15	Psychoneurosis
					5.4 5.0	82	110-91	231	11	5.4		15	
					4.4 3.7	68	110-95	213	13	4.9		15	
					3.9 4.6	62	112-95	191	11	3.8		15	
					4.0 4.4	72	104-70	182	19	5.3	437	15	
					4.1 4.1	69	100-75	173	16	4.7		15	
MORPHINE (CARDIAC CASES)													
245	M.	57	69	157	3.5 3.7	46	149-77	212	12	5.1	477		Coronary occlusion. Angina pectoris
246	M.	33	66	176	3.8 3.5	51	141-72	193	11	4.4		15	Angina pectoris
247	M.	43	70	181	0.0 5.2	86	150-90	247	16	5.4	700	15	Hypertensive heart disease. Just out of decompensation
248	M.	65	67	140	4.1 5.1	82	145-85	250	11	4.8		15	Thyroid heart disease. Auricular fibrillation. Never decompensated
249	M.	51	66	108	3.5 2.9	75	165-99	233	20	7.8	1240	15	Arteriosclerotic heart disease. Aortic stenosis (?)
250	M.	39	67	119	3.0 4.0	68	180-100	280	15	6.4		30	Attacks of nocturnal dyspnea
251	M.	59	63	178	4.2 4.4	98	124-65	305	14	7.3	1282	30	Rheumatic heart disease. Decompensated 6 months ago.
301	M.	22	67	149	4.0 3.3	88	113-65	270	14	6.0		30	Mitral stenosis
					3.5 3.4	72	128-85	239	17	6.8	1345	30	Coronary thrombosis 1 month before
					2.8 3.1	74	129-65	241	13	5.4		30	Hyperthyroidism
					2.9 2.4	78	115-82	206	20	6.0	1380	30	
					2.1 2.6	79	127-72	257	22	5.5	585	30	
					2.3 2.7	77	109-67	229	19	5.4	638	30	
					7.5 6.3	102	129-59	400	14	8.4	799	15	
					5.6 6.8	104	132-59	440	14	8.2	644	15	
STRYCHNINE													
118	F.	47	61	153	2.0 1.4	80	103-69	162	20	4.5	1083		(?) Rheumatic heart disease. Very few physical signs. On digitalis
291	M.	22	65	132	1.5 1.7	60	95-62	143	23	4.5	1105	2.0	Rheumatic heart disease. Mitral stenosis. Minor pulmonary hemorrhage
292	M.	14	66	129	2.5 2.9	55	119-71	205	20	6.6	1100	1.5	Recent rheumatic fever
294	M.	28	65	134	2.5 2.5	54	123-72	165	15	5.6	990	1.0	(?) Cardiac involvement
295	M.	30	68	144	5.6 5.1	72	116-68	222	11	5.6	510	1.5	Rheumatic heart disease. Advanced aortic regurgitation with all peripheral signs
296	M.	27	70	152	4.0 3.6	64	120-64	208	10	4.8	464	2.0	Thromboangiitis obliterans
297	M.	51	64	160	4.8 4.1	52	127-24	250	13	6.2	1500	1.5	Thromboangiitis obliterans
316	M.	48	68	171	4.0 3.6	54	133-83	199	14	5.9	672	1.5	Heart enlarged without obvious cause
					3.0 3.1	55	132-76	217	14	5.7	548	2.0	Nervous. Minor subject
					4.5 4.2	59	98-66	256	8	8.5	669	3.0	Arteriosclerotic heart disease. Auricular fibrillation. On digitalis
					6.5 5.7	98	109-85	258	20	11.6	1277		
					5.0 4.1	90	110-85	220	19	8.8	1170		
					3.3 2.6	80	160-115	287	12	6.0	986		
					2.7 2.3	80	152-110	284	15	6.2	991		

and it appeared from 15 to 45 minutes after the subcutaneous injection.

The action of ephedrine was rather irregular; some of our subjects responded markedly, others very little, to the same subcutaneous dose. Our results show a significant increase in pulse rate, systolic blood pressure, respiratory volume, cardiac output and cardiac work, and a significant diminution of peripheral resistance. The pattern of the response (Figure 3) closely resembles that

found after adrenalin, but ephedrine is less powerful in the dosage given. The stimulant action of ephedrine on the heart is also less than that of adrenalin (Figure 1).

The increase in metabolic rate was irregular, being marked in some subjects, absent in one. This seems to have been the experience of others also (41).

The electrocardiograms of all but one of our subjects showed diminution in height of the T



wave after the drug. In Case 310, who had been clinically much improved by the drug, an upright T wave became biphasic. Changes of this type have been reported on dogs and in an occasional patient after the drug (42).

The action of ephedrine, a synthetic racemic ephedrine, was carefully studied in a single normal subject by Euler and Liljestrand (43). We used the natural *l* rotatory ephedrine which is more active than the racemic. Therefore, our results are not strictly comparable with those of Euler and Liljestrand, but they agree as well as one would expect.

The depressant action of ephedrine on the heart, demonstrated after large doses in animal experiments (42) does not seem to be a factor in the clinic after proper dosage of the drug.

As is the case with epinephrine the diminution in peripheral resistance indicates that vessels have dilated somewhere and that the increased blood pressure is to be entirely explained by increased cardiac output. Such a large increase of cardiac output accompanied by general vasoconstriction would surely cause a dangerous rise of blood pressure. Doubtless the nervous and humoral mechanisms of homeostasis, brought into action by the elevation of pressure, overbalance the constrictor action of the drug.

#### *Caffeine and theophylline ethylenediamine*

These drugs, belonging to the same group, may be considered together. Both drugs, although injected well into the deltoid muscle gave rise to soreness at the site of injection in every instance. Both drugs had but little effect on the functions usually measured in the clinic—pulse rate, respiratory rate and blood pressure—so that we could do little more than guess at the time of maximum drug effect. We made the second group of estimations from 20 to 40 minutes after the injection.

The averages show a marked increase in cardiac output, work and respiration; and a diminution of peripheral resistance. These changes are significant for theophylline. Figure 3 shows that the average circulatory and respiratory effects of the two drugs have a similar pattern, theophylline being more powerful in the dosage used. Both drugs are cardiac stimulants (Figure 1).

The electrocardiograms of our patients showed no noteworthy change after either drug.

Three of the seven patients given theophylline had angina pectoris. In spite of the increased heart work demonstrated in these cases no cardiac pain followed in any instance.

Our results after intramuscular injections of caffeine correspond in a general way with those obtained by Grollman (6) after giving caffeine by mouth to normal persons. The effect of caffeine on the metabolic rate of our patients is similar to the experience of others (6, 41) in that, while most subjects show no change after the drug, an occasional person shows a marked increase.

In three cases of congestive heart failure, one studied three times, Friedman, Resnik, Calhoun and Harrison (44) made single estimations of cardiac output by the acetylene method before and after the oral administration of from 1.5 to 0.9 grams of theophylline daily for two days. The average of the results showed an increased cardiac output of 9.8 per cent after the drug but the results of individual experiments were irregular and this difference, judged by the standards we have set for ourselves, is not significant. These investigators were chiefly interested in the effect on the circulation of loss of fluid by diuresis, and so they had no need of performing their estimations at the height of drug action. We believe, therefore, these results to be as similar to ours as should be expected.

Our studies indicate that these two drugs rank among the powerful cardiac and circulatory stimulants. We believe that clinicians, basing judgment on their lack of effect on pulse rate, respiratory rate and blood pressure, have underestimated their activity, and possibly their value also.

#### *Carbaminoylcholine*

Carbaminoylcholine (doryl) was investigated because of the especial interest of one of the authors (45). It is a representative of the group of drugs whose action is similar to that which follows stimulation of parasympathetic nerves. Of the 6 patients, 4 were subject to peripheral vascular disease and previously had had relief of pain after the drug. All of them had improved to the extent of freedom from pain when these tests



were made. A slight flush with some diminution of blood pressure and increase of pulse rate gave evidence that the drug was acting when the estimations were made, 20 to 40 minutes after the subcutaneous injections.

The average results, Figure 3, show a significant increase of pulse rate and respiration, and a significant diminution of blood pressure and peripheral resistance. The average cardiac output increased 12 per cent but the changes found in individual cases were irregular enough to prevent significance being attained in so small a series.

When plotted in Figure 1 the average results do not indicate any stimulant or depressant effect on the heart.

The electrocardiograms showed no change of form which could be attributed to the drug.

After doses of the size given the effects on the circulation are much similar to those which follow other vasodilators. We find no evidence of any effect like cardiac vagus stimulation.

### *Nitrites*

All but one of the patients tested had a pronounced hypertension. A dose of 0.06 gram of sodium nitrite in solution was given by mouth as soon as the control estimations were over, and repeated about 30 minutes later just before the second inhalation of ethyl iodide began. The change in blood pressure guided us in making the two estimations of cardiac output at the height of the action of the drug. This was usually about 20 or 30 minutes after the second dose.

Case 280 was obviously excited during the control period and its data, included in Table III, have been omitted from the statistical analysis. The results show that a diminution of blood pressure occurred in all but one case, which, although given a third dose, still showed no noteworthy change. The average diminution of blood pressure and peripheral resistance are significant. The average heart work diminished with the fall in blood pressure, but there was enough variation in the individual cases to prevent significance being attained. The averages of the cardiac output and of the other functions measured were not materially influenced by the drug (Figure 3).

The electrocardiograms of Case 280 showed a surprising change. In Lead II the T wave, up-

right less than 1 mm. before the drug, increased to 6 mm. after it; in Lead III the T wave, originally inverted 1 mm., became 4 mm. upright. The electrocardiogram during the drug's action was far more normal than that taken before it. Cases 318 and 322 showed very slight increases in height of the T wave in Lead II during the action, but Case 319 showed a 1 mm. diminution of the T wave in this lead. The electrocardiograms of the other patients were unchanged. The changes found are similar to those described by Evans and Hoyle (46) after amyl nitrite and nitroglycerine.

Our results, Figure 1, indicate that sodium nitrite causes slight depression of the heart, a finding consistent with the results of animal experiments (22).

Sodium nitrite gives effects enduring long enough to permit duplicate estimations of cardiac output during its action. Therefore it was preferable for this investigation to the rapidly acting nitrites whose action may change materially in intensity before such estimations can be completed. However, in two cases of hypertension we obtained single estimations of cardiac output during the period of diminished blood pressure following the administration of nitroglycerine (Table III).

After a single control estimation, tablets totalling 1.2 mgm. of nitroglycerine were placed under the tongue. In the first case the pressure diminished sharply, attaining a minimum 10 minutes after the administration of the drug. A marked increase in cardiac output was present at that time. Forty minutes later, 20 minutes after the blood pressure had returned to its previous level, the estimated cardiac output had fallen, but not to the control level. In the second case, after a slow fall, the systolic blood pressure curve became level and did not regain its previous height during the next 35 minutes. Twenty minutes after the administration of the drug the cardiac output was but little elevated. Fifteen minutes later it was markedly increased. Obviously, there was a significant increase in cardiac output after nitroglycerine in these two cases, and the extent of the change was larger than that usually seen after sodium nitrite.

In general our results are similar to those of our predecessors in this field. Weiss and Ellis

(47) studied 5 cases of hypertension employing the cardiac output method of Field, Bock et al. which gives higher results than the more recent methods. After doses of sodium nitrite larger than those we employed, the average cardiac output diminished 12.6 per cent. If one omits one of their cases with an abnormally high output before the drug, the diminution is only 7 per cent, in contrast to an increase of 3.4 per cent in our series. Neither change is significant and in other respects our results resemble theirs.

Gaisböck and Jarisch (48) using the Krogh and Lindhard method repeatedly on two normal subjects, found an average increase of 44 and 20 per cent in cardiac output after subcutaneous injection of small doses of sodium nitrite although the pulse rate and blood pressure were unchanged. Weiss and Ellis (47) found no change in cardiac output of normal persons after large doses by mouth. On four normal persons Lindhard (49) found a significant increase of cardiac output, averaging 23 per cent, after acute experiments with amyl nitrite. In the same subject, more prolonged inhalation was followed by a smaller effect which, judged by our standards, was not significant.

Lauber and Brauch (39) calculating the cardiac output from the pulse wave form, velocity, and pressure, according to Broemser's method, divide the action of nitroglycerine into two parts. At first output and work are increased, later both are diminished.

In general the results indicate that when the blood pressure is lowered rapidly by a nitrite the cardiac output is regularly increased. If the blood pressure falls slowly or not at all the results are less regular, either an increase or no change being found. In our series cardiac work per minute diminished, not significantly, after sodium nitrite but apparently increased in the two experiments after nitroglycerine.

#### *Pitressin*

We made four observations of pitressin action on three patients with diabetes insipidus who were accustomed to take the drug, and on three other patients who had never received it. In the former, pituitary preparations were withdrawn for about 18 hours and polyuria had developed

before the control estimations. These patients' urine was measured before and after they received their usual dose at the time of testing. The polyuria was checked in every instance. Estimations were made 20 to 50 minutes after the drug's administration, usually by subcutaneous injection. Marked facial pallor gave evidence that the drug was acting when the estimations were made.

The results, Table III, do not demonstrate any obvious difference between the response of the patients with diabetes insipidus and the other patients, therefore both sets have been averaged in Table I and Figure 3. The results on Case 293, who inhaled pituitary powder, have been omitted from the statistical calculations because of lack of knowledge of the dose absorbed.

The averages show that no significant change in any item measured followed pitressin. Most of the individual results show the same thing. Case 260 showed a marked diminution of cardiac output after the drug but a second test did not confirm the finding. Case 293 alone showed a decided increase.

The electrocardiograms were unchanged after the drug except in Case 328 whose P waves diminished from 2 to 1 mm. in height in Leads II and III after the drug. We did not observe any prolongation of A-V conduction time as has been observed both in animals and man (52).

When plotted in Figure 1, our averages do not indicate that any depression of the heart resulted from these therapeutic doses of the drug. That relatively larger doses depress the heart in heart-lung and other animal preparations is well known (23).

Pituitrin is known to produce inconstant changes of blood pressure in different patients. Moffat (51) studied 62 cases; in the majority there was little or no change after 1 cc. intramuscularly. The general trend was towards a slight rise immediately after injection, and downward thereafter. Our results with pitressin are quite similar.

Grollman and Geiling (52) studied two normal subjects by the acetylene cardiac output method. Therapeutic doses of pituitrin and pitressin produced a transient diminution of pulse rate, oxygen consumption, and cardiac output, followed by a more prolonged increase. Hartl (53), calculating the cardiac output from blood pressure curves

and pulse wave velocity according to Broemser's method, deduced an increased peripheral resistance and a diminished cardiac output after 10 units of pitressin in one subject. The curve of these results resembles that of Grollman and Geiling but the changes are not so marked and the secondary rise of cardiac output did not reach the initial level.

Our estimations of pulse rate, cardiac output and metabolism, made during the period after administration of the drug in which Grollman and Geiling obtained evidence of an increase of these functions, did not give similar results.

We have not investigated the changes in cardiac output found soon after the injection by the ethyl iodide method, but Dr. H. A. Schroeder made estimations by the acetylene method 10 minutes after the injection of 20 units of pitressin in three patients. The average output was diminished 11 per cent at this time, as found by Grollman and Geiling, but unlike their results, the metabolic rates were essentially unchanged. Varying effects of pituitrin on metabolic rate have been reported by numerous authors (52). It is of interest that in our results the changes average out.

We conclude that pitressin is a drug usually causing little enduring change in the functions we have measured. The more pronounced effects, seen occasionally, vary in their direction so that average changes are not significant.

### *Quinidine*

The quinidine was administered by mouth and the subsequent observations were begun as soon as the action manifested itself by an increase of pulse rate, usually between one and two hours after the drug had been taken. The dosage was larger than that usually given in a single dose in the clinic. Case 285 probably received a slight overdose for she vomited after the estimations although she denied nausea during them.

Two of the cases had auricular fibrillation which persisted in spite of the drug. Two had previously been digitalized and the only instance of diminished pulse rate after quinidine occurred in one of these. In other respects the action of quinidine after digitalis did not seem different.

All the changes demonstrated after quinidine were small but some occurred with such regularity

that they were significant. The average pulse rate rose significantly. The stroke volume and work per beat were significantly diminished (Table I, Figure 3).

The average size of the heart diminished significantly and this may well be a reflection of the change in heart rate. A more rapid rate, providing less time for filling, must surely reduce the diastolic size if the inflow remains unchanged.

The electrocardiograms were changed in all cases with normal rhythm, the T wave being from 1 to 2 mm. more upright in two or three leads after the drug. This was very striking in Case 282. In Case 285, Lead III, the QRS complex changed its form, the Q wave originally present disappearing, and the complex becoming M shaped. These results differ from those usually described, for, after patients have been given the drug daily for several days, inversion of the T wave is the common finding (50). In anesthetized dogs soon after intravenous injection of quinidine Lewis et al. (54) described an elevation of the T wave probably analogous to that seen in our acute experiments.

By means of animal experiments quinidine has been proved a strong cardiac depressant. The average results do not demonstrate cardiac depression in our subjects, although they received larger doses than usual. Perhaps clinical experience has set the dosage of quinidine at a point where cardiac depression is minimal.

### *Morphine*

This drug was the first studied, and our data are less complete than in other groups. The size of the heart after the administration of the drug was estimated in only four cases, the electrocardiogram was taken in but one.

Seven determinations were performed on patients without heart disease and not seriously ill. The estimations during morphine action were made as soon as pupillary constriction was manifest, and were performed between  $\frac{1}{2}$  and  $1\frac{1}{2}$  hours after the subcutaneous injection. The averages (Table I and Figure 3) show little change in heart or circulation. The largest change was in the average pulse rate which diminished about 5 per cent, but even this did not attain signifi-

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# THE OPSONO-CYTOPHAGIC REACTION OF THE BLOOD IN PERTUSSIS<sup>1</sup>

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Investigations of humoral immunity in pertussis have been chiefly directed toward studies relating to specific agglutinins and complement-fixing antibodies. One direction which recent work has taken concerns the study of the opsonocytophagic reaction of the blood. As the name implies, this reaction is a test for the opsonizing antibody in the serum as well as for the phagocytosing power of the leukocytes. Whole blood may be used, as pointed out by Veitch (1), and this method has been successfully applied by Huddleson and his coworkers (2) in undulant fever. The results of their studies stimulated us to investigate the reaction in pertussis, particularly in regard to the therapeutic effect of intravenously or intramuscularly injected immune blood (3). While the work was in progress, it was learned that Kendrick (4) had reported observations on the reaction made during the course of pertussis, and in animals before and after injection of suspensions of *H. pertussis* and of other organisms. She suggested that the reaction might be useful in studying immune processes in pertussis.

Singer-Brooks and Miller (5) have recently described their experience with the reaction and have presented the results obtained from observations made during the course of the disease. They have been particularly interested in the reaction of vaccinated children.

This report deals with observations of the reaction in children during the course of pertussis; in children with positive and with negative histories of the disease; and in mothers and their newly-born infants.

<sup>1</sup> This investigation was aided, in part, by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

<sup>2</sup> Working under a grant from the Fluid Research Fund of the University of Rochester.

## METHODS AND MATERIALS

Freshly isolated strains of *H. pertussis* were used to produce antigens. In each instance they were found to be hemolytic on the modified Bordet-Gengou medium; were morphologically of the coccoid-bacillary type; were virulent for mice; produced necrotic lesions when injected intradermally into guinea pigs in dosages of 0.05 cc. of a suspension containing one billion organisms per cc.; and were agglutinated by Phase I antisera but not by Phase IV antisera. Accordingly, by these criteria, the strains were of the virulent or Phase I type.

When the strains revealed any change from Phase I characteristics, their use was discontinued and newly isolated ones were substituted. Antigens were prepared every two to four weeks and were kept in the ice box at 4° C. during the period used.

The medium used consisted of (a) a potato-glycerine-agar base, and (b) defibrinated sheep's blood.

The base consisted of the following ingredients: peeled potato, 500 grams; glycerin, U.S.P., 40 cc.; Bacto-agar, 120 grams; sodium chloride, C.P., 21.5 grams; distilled water, 4,000 cc.

It was prepared as follows. The water and glycerin were mixed. The sliced potatoes were wrapped in gauze and suspended in one-half the volume of water and glycerin mixture, and boiled until soft. The potatoes were removed and the liquid allowed to cool. The salt and the agar were then added and the mixture allowed to stand for 15 minutes in order to saturate the agar. The mixture was then heated to dissolve the agar. The remaining water-glycerin mixture was added and the mixture brought up to volume.

The base mixture was dispensed into flasks, each containing 150 cc., and autoclaved for 25 minutes at 15 pounds pressure.





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The base mixture was dispensed into flasks, each containing 150 cc., and autoclaved for 25 minutes at 15 pounds pressure.

When a fresh batch of medium was desired, a flask containing 150 cc. of this base was melted in a water bath, cooled to 45° C. and to this amount, 30 cc. of freshly drawn defibrinated sheep's blood was added. Plates or slants were then poured as needed.

This medium was found to give satisfactory results after storage in the ice box for several days, but each batch of plates or tubes was dated and at the end of three days, the unused supply was discarded.

For the growth of subcultures from which the antigens were prepared, Bradford's gonococcus medium (6) was frequently used. This medium was prepared by melting 100 cc. of 2 per cent Douglas agar (pH 7.6 to 7.8). When this base had been cooled to 45° C., 25 cc. of the sterile ascitic or hydrocele fluid, 5 cc. of sterile 20 per cent glucose, and 10 cc. of sterile defibrinated rabbit's blood were added and mixed. Plates were poured in the usual manner.

Upon this medium the majority of freshly isolated strains of *H. pertussis* grew well after a few generations of growth upon the modified Bordet medium.

The technique of determining the opsono-cytophagic reaction consisted of mixing 0.05 cc. of whole blood, obtained as it flowed freely from a small incision in the finger tip, with 0.05 cc. of a 1 to 1,000 solution of heparin in physiological salt solution. To this was added 0.05 cc. of a standard killed suspension of Phase I *H. pertussis*. This suspension was made by scraping a forty-eight hour growth of the organism from Bradford's medium into physiological salt solution. Merthiolate was added to make a final concentration of 1 to 10,000, and the suspension was standardized to match the turbidity of a known standard suspension containing approximately ten billion organisms per cc.

The organisms were added within thirty minutes after the blood was withdrawn, the mixture was shaken and placed in a 37° C. water bath for 30 minutes. A second shaking was done after 15 minutes of incubation. At the end of the 30 minute period, without further shaking, smears were made, fixed with methyl alcohol, stained by the Giemsa method for 20 minutes, washed, dried, and examined under oil immersion.

A series of 25 consecutive polymorphonuclear leukocytes were examined and the organisms engulfed in each counted. This number of cells was selected because it was observed that the final results were not significantly altered when series of 50 or of 100 cells were counted. The cells were then classified according to the number of organisms engulfed. Three arbitrary groups were used to denote the degree of phagocytosis as follows: "none to slight," 0 to 4 organisms; "definite," 5 to 19 organisms; "marked," 20 or more organisms. If a leukocyte was distorted or if the organisms could not be counted because of marked clumping within the cytoplasm, the cell was omitted from the series. It was noted that phenolized antigens gave lower degrees of phagocytosis than did the merthiolate-preserved ones. There was no significant difference between the degree of phagocytosis obtained when living and when merthiolate-killed antigens were tested, or when the virulent Phase I and the avirulent Phase IV antigens of the same strain were compared.

## RESULTS

The reaction of the blood of 49 patients, when tested at various periods during the course of the disease, is shown in Table I. For each group

TABLE I  
*The opsono-cytophagic reaction of the blood during the course of pertussis*

Week of disease	Number of patients tested	Distribution of the average number of cells according to the number of organisms phagocytosed		
		0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
1 and 2.....	13	2.8	18.0	4.2
3 and 4.....	15	1.3	14.5	9.2
5 and 6.....	12	0.8	9.5	14.6
7 and 8.....	9	0.6	8.6	15.7

tested, the distribution of the average number of leukocytes according to the number of organisms phagocytosed is recorded. It is obvious that, as convalescence approaches, there is a definite increase in the number of cells in the "marked" column and a corresponding decrease in the number of cells found in both the "none to slight" and "definite" groups.

TABLE II

*The opsono-cytophagic power of the blood during the course of pertussis*

Patient	Age	Week of disease	Distribution of cells according to the number of organisms phagocytosed		
			0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
Ru.....	15 months	1st	3	22	0
		2nd	0	21	4
		3rd	0	0	25
		4th	0	14	11
		5th	0	5	20
Br.....	18 months	2nd	0	19	6
		3rd	0	19	6
		4th	1	20	4
		5th	1	9	15
McStr...	2 months	2nd	0	23	2
		5th	0	15	10
		6th	0	1	24
		7th	0	8	17
O'C.....	2 years	3rd	0	12	13
		5th	3	14	8
		6th	0	2	23
		7th	0	13	12
Ka.....	9 months	8th	0	3	22
		4th	1	24	0
		6th	1	3	21
Ho.....	6 years	7th	0	4	21
		4th	0	10	15
		5th	0	12	13
		7th	0	3	22

TABLE III

*The degree of phagocytosis in relationship to the history of the disease*

Age	Number tested	Distribution of the average number of cells according to the number of organisms phagocytosed		
		0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
NEGATIVE HISTORY OF PERTUSSIS				
6 weeks to 6 months...	8	8.1	13.6	3.2
6 months to 2 years...	16	3.5	16.9	4.6
2 to 5 years .....	7	1.7	11.9	11.4
5 to 15 years .....	7	1.7	14.3	9.0
POSITIVE HISTORY OF PERTUSSIS				
6 weeks to 6 months...	1	0	8	17
6 months to 2 years...	5	0.2	6.2	18.6
2 to 5 years .....	4	1.0	11.5	12.5
5 to 15 years .....	20	0.2	8.4	16.3

Table II illustrates a similar increase in the phagocytosing power of the blood when tested on the same patient during the disease.

In Table III, the results obtained from studying a group of 38 infants and children with negative histories of the disease are compared with those obtained in a group of 30 who gave positive histories. It is apparent that, in general, the degree of phagocytosis is greater in the blood of those who had the disease, particularly in those under 2 years of age. In the older children (2 to 15 years) with negative histories, a high degree of phagocytosis was often observed. This suggests that the test is not entirely dependent upon

TABLE IV

*A comparison of the opsono-cytophagic reaction of the blood in twenty mothers and their newborns in relationship to the history of pertussis in the mother*

Name	Distribution of the polymorphonuclear neutrophils according to the number of organisms phagocytosed					
	Mothers			Newborn		
	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
POSITIVE HISTORY OF PERTUSSIS						
Si.....	0	2	23	0	4	21
McB.....	0	12	13	5	20	0
Wa.....	1	10	14	0	17	8
Ha.....	0	13	12	16	9	0
Er.....	0	7	18	24	1	0
Fe.....	0	4	21	2	6	17
Ba.....	0	6	19	0	12	13
Ta.....	0	4	21	1	10	14
Average number of cells	0.1	7.2	17.6	6.0	9.9	9.1

NEGATIVE HISTORY OF PERTUSSIS

Sm.....	1	12	12	8	12	5
Co.....	0	25	0	3	22	0
La.....	1	21	3	8	17	0
Wa.....	0	2	23	10	15	0
Sp.....	0	11	14	14	10	1
Sl.....	0	17	8	3	12	10
Do.....	0	20	5	7	14	4
Fe.....	0	15	10	10	15	0
Pe.....	2	17	6	18	7	0
Ki.....	0	20	5	11	14	0
Sh.....	2	22	1	6	19	0
Le.....	0	0	25	0	6	19
Average number of cells	0.5	15.1	9.3	8.1	13.5	3.3

specific antibodies, for it is well known that, within certain limits, normal opsonins increase with age. Moreover, we have observed increased titers in the blood of febrile patients, and in patients after artificial heat therapy. These results probably depend, in part at least, upon a temporary increase in the so-called normal opsonins. Singer-Brooks and Miller (5) have observed similar results after the administration of nonspecific "respiratory" vaccines.

In Table IV, results of tests upon 20 mothers and their infants during the first 10 days of life are shown. It is apparent that, in general, the titer of the mother's blood is greater than that of the offspring and that a high titer in the newborn is almost invariably associated with a high titer in the mother. A high titer in the newborn is almost never associated with a low titer in the mother. This suggests that placental transfer of the opsonizing antibody occurs. Further investigation of this finding is in progress. When the averages of the cell distribution are compared, it is noted that the degree of phagocytosis is greater in the bloods of both mother and baby when there is a history of the disease in the mother.

#### SUMMARY

The opsono-cytophagic power of the blood increases during the course of pertussis. It is generally greater in those who have had the disease than in those who have not. Evidence is presented to suggest placental transfer of the antibody in certain instances.

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# THE EFFECT OF IMMUNE BLOOD UPON THE OPSONO-CYTOPHAGIC POWER OF THE BLOOD IN PERTUSSIS<sup>1</sup>

By WILLIAM L. BRADFORD, ROBERT MIKELL AND BETTY SLAVIN<sup>2</sup>

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(Received for publication April 26, 1937)

In comparison with the general renewal of interest in vaccine prophylaxis in pertussis, little attention has been given to passive protection by the use of immune blood. There seems to be sufficient evidence to indicate that this method is effective in the prevention and modification of the disease in infants. It is probable that the very young infant does not respond well to antigenic stimulation. Moreover, in the case of the exposed infant, the time interval may not permit a satisfactory application of active immunization. Clinical results obtained by the use of adult immune serum and convalescent serum in the prevention of whooping cough, along with a review of the literature, have been described (1). In this report an attempt to measure the effect of these agents in terms of humoral antibodies is made.

Since recent clinical and experimental evidence strongly favors *H. pertussis* as the cause of whooping cough, and since definite proof of a circulating soluble toxin in the disease is lacking, it is justified to assume that recovery depends to a great extent upon phagocytosis. This process has long been appreciated as important in the general mechanism whereby the host rids itself of infecting bacteria.

In the past, investigation of humoral immunity in pertussis has chiefly concerned agglutinins and complement-fixing antibodies. These antibodies appear at the beginning of the third week of the disease and diminish significantly as convalescence reaches the fifth or sixth month. They are demonstrable in the blood for a similar period of

time following active immunization with *H. pertussis* vaccine.

Recent attention has turned to the application of the opsono-cytophagic test, suggested by Veitch (2), and used by Huddleson et al. (3) in undulant fever, for studying immune processes in pertussis. As the word implies, this is a test for the opsonizing antibody of the serum as well as for the phagocytosing power of the leukocytes. Whether phagocytosis, as demonstrated by this method, is simply another way of studying an antibody identical with agglutinins, precipitins and complement-fixation substances, depends upon one's interpretation of the unitarian theory of antibodies and is a question which is beyond the scope of this report.

The technique of the test and the materials used have been described in the preceding paper (4).

By the use of this test, which is probably not an entirely specific reaction, we have observed that the degree of phagocytosis of *H. pertussis* is increased in the blood during the course of whooping cough. Children who have had the disease have shown higher titers than those who have not and mothers have had, in general, higher titers than have their newborn infants. We have likewise collected evidence that suggests placental transfer of the opsonizing antibody (4).

## RESULTS

In Table I, the results obtained by adding immune adult serum to the whole blood-heparin-organism mixture in each of 11 individuals are shown. In these tests 0.05 cc. of the patient's blood obtained from a puncture of the finger tip was mixed with 0.05 cc. of heparin (1 to 1000 dilution in physiological salt solution). To this was added 0.05 cc. of the standard antigen (10

<sup>1</sup> This investigation was aided, in part, by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

<sup>2</sup> Working under a grant from the Fluid Research Fund of the University of Rochester.

TABLE I

*The effect of adult immune serum upon phagocytosis of H. pertussis in vitro*

Patient	Age	Distribution of polymorphonuclear neutrophils according to the number of organisms phagocytosed					
		Saline (control)			Serum		
		0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
Su.....	18 months	0	13	12	0	1	21
Ro.....	7 years	3	15	7	0	0	25
Ed. J.....	2 years	0	16	9	0	4	21
Ed. W.....	5 years	0	18	7	0	3	22
Ro.....	Newborn	0	14	11	0	9	16
Sm.....	Newborn	1	22	2	0	11	14
Ru.....	15 months	3	22	0	0	3	22
Cl.....	4 weeks	0	8	17	0	4	21
Jo.....	Newborn	7	9	9	5	10	10
Fa.....	Newborn	0	14	11	2	20	3
Ja.....	Newborn	1	22	2	0	15	10
Average number of cells.....		1.4	15.7	7.9	0.6	7.3	17.1

billion organisms per cc. in physiological salt solution preserved by a 1 to 10,000 concentration of merthiolate) and 0.05 cc. of the immune adult serum. This serum was that of adults who had previously had the disease in childhood. In the controls 0.05 cc. of physiological salt solution was substituted for the adult immune serum. It is obvious that in all except one instance the number of cells exhibiting marked phagocytosis is definitely increased as compared with the respective saline control.

Table II shows the results obtained when a similar comparison is made between the influence of the serum of an individual before, and 48 hours after, receiving an intravenous blood transfusion. In this experiment, this influence was determined upon the leukocytes of two newborn infants in each case. The blood of newborn infants was

TABLE II

*The effect of transfusion upon phagocytosis of H. pertussis*

Patient receiving transfusion	Age	Cells of newborn infants	Distribution of polymorphonuclear neutrophils according to the number of organisms phagocytosed											
			Saline control			Serum of patient before transfusion			Serum of patient after transfusion			Serum of donor		
			0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
Cl.	4 weeks	a b	0 1	14 22	11 2	2 0	20 15	3 10	0 0	10 8	15 17	0 0	9 11	16 14
St.	2 years	a b	23 6	2 17	0 2	23 6	2 16	0 3	0 0	16 20	9 5			
Sp.	5 years	a	3	12	10	1	16	8	0	13	12	0	7	18
He.	8 weeks	a b	3 5	19 20	3 0	6 13	19 12	0 0	5 12	20 8	0 5			
Ku.	2 years	a b	3 5	18 20	4 0	13 10	12 15	0 0	3 0	22 23	0 2			
D'A.	12 months	a b	8 23	8 2	9 0	0 13	10 10	15 2	1 7	7 10	17 8			
Co.	18 months	a b				10 19	12 5	3 1	11 5	10 11	4 9	10 17	7 6	8 2
Ke.	6 years	a b	9 7	16 17	0 1	2 0	23 25	0 0	3 0	21 23	1 2	5 1	14 17	6 7
Ca.	5 months	a b	0	17	8	2 11	20 13	3 1	0 8	24 17	1 0	0 1	15 24	10 0
Average number of cells			6.8	14.5	3.6	7.7	14.4	2.9	3.2	15.4	6.3	3.7	12.2	9.0

TABLE III

*The effect of intramuscular injection of 20 cc. of adult whole blood upon phagocytosis of H. pertussis*

Patient receiving transfusion	Age	Cells of newborn infants	Distribution of polymorphonuclear neutrophils according to the number of organisms phagocytosed											
			Saline control			Serum of patient before transfusion			Serum of patient after transfusion			Serum of donor		
			0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
Ru.	15 months	a b	14 0	2 2	9 23	9 14	6 3	10 8	11 7	4 3	10 15			
McS.	2 months	a	8	12	5	11	14	0	5	16	4	1	18	6
Fe.	12 months	a b	2 7	22 18	1 0	0 2	23 22	2 1	0 2	25 21	0 2	2 0	22 24	1 1
DeC.	18 months	a b	3 15	21 10	1 0	0 16	20 9	5 0	0 3	25 21	0 1	0 0	18 24	7 1
Ta.	3 years	a b	4 17	20 8	1 0	0 2	13 17	12 6	1 1	17 9	7 15	0 0	12 15	13 10
Average number of cells			7.7	12.7	4.4	6.0	14.1	4.9	3.3	15.7	6.0	0.4	19.0	5.6

selected in order to eliminate the possibility of the effect of an unrecognized attack of pertussis, and because it was easier to obtain low titers as controls. Tests of the effect of the addition of normal saline as well as of the donor's serum are included for comparison.

It is evident that serum taken after the transfusion stimulated the leukocytes of the newborn infant to a greater degree of phagocytosis than did the serum taken before the transfusion. The donor in each instance was a parent whose history of having had the disease was not determined. The amount of blood given in each case was approximately 10 cc. per kilogram of body weight.

In Table III, the effects of serum samples taken before and after the intramuscular injection of 20 cc. of whole blood into each of 5 infants is recorded. The donor in each instance was a parent whose history of the disease was not known. While the effect upon phagocytosis is not as marked as in the case of those receiving transfusions, it is apparent that a definite increase has occurred.

Table IV includes results obtained by the intramuscular injection of 10 cc. of hyper-immune human serum. In this experiment the whole blood

TABLE IV

*The effect of the intramuscular injection of 10 cc. of hyper-immune adult serum upon phagocytosis of H. pertussis*

Patient	Age	Distribution of polymorphonuclear neutrophils according to the number of organisms phagocytosed					
		Before injection			After injection		
		0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
Fa.....	3 months	0	21	4	0	21	4
Cr.....	10 months	10	15	0	0	25	0
Ta.....	18 months	1	18	6	0	13	12
St.....	2 years	0	4	21	0	13	10
Br.....	9 months	0	19	6	0	19	6
Fo.....	3 weeks	5	20	0	5	19	1
McS.....	2 months	5	18	0	0	21	4
Mi.....	Newborn	10	15	0	4	21	0
Mit.....	Newborn	19	6	0	22	3	0
Ro.....	Newborn	2	21	2	7	18	0
Vi.....	Newborn	14	11	0	9	14	0
Ex.....	Newborn	10	15	0	0	25	0
Ec.....	Newborn	19	4	2	0	15	10
Average number of cells.....		7.3	14.4	3.3	3.6	17.6	3.8

of the recipient before and after receiving the injection was tested directly. The hyper-immune serum was prepared by immunizing normal young adults by three weekly injections of Phase I *H. pertussis* antigen, each injection representing five

billion organisms. The donor was bled two weeks after the final injection, the serum harvested and preserved by the addition of 0.25 per cent tricresol. Kendrick (5) has reported excellent prophylactic results from the use of similar serum in exposed infants. The increase in phagocytosis brought about by this serum is not as great as that produced by transfusion, but consists chiefly in a shift from "none to slight" to "definite."

The effect of injecting 10 cc. of placental extract was studied in 3 infants and, as shown in Table V, a definite increase in the phagocytic

TABLE V

*The effect of intramuscular injection of 10 cc. of placental extract upon phagocytosis of H. pertussis*

Patient	Age	Distribution of polymorphonuclear neutrophils according to the number of organisms phagocytosed					
		Before injection			After injection		
		0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
	months						
McS.	2	0	14	11	1	7	17
So...	3	2	13	10	0	8	17
Ka...	9	1	24	0	0	15	10
Mi.*	2	5	16	4	0	8	17

\* Received 20 cc. immune rabbit serum.

power of the blood resulted. In 1 infant, who received 20 cc. of immune rabbit serum, a similar response occurred. This immune rabbit serum was prepared by injecting rabbits intravenously with *H. pertussis* vaccine made from Phase I organisms; standardized to contain ten billion organisms per cc., and killed with merthiolate (1 to 10,000). Three weekly injections of 0.5 cc., 1.0 cc., and 1.5 cc. of the vaccine were given, and the rabbit was bled ten days after the last injection. The serum was separated and preserved by the addition of 0.25 per cent tricresol.

## SUMMARY

The opsono-cytophagic reaction of the blood has been used to test the effect of the injection of immune blood in pertussis.

In 10 of a group of 11 infants and young children, the blood in each instance showed a marked increase in phagocytic power against *H. pertussis*, when a small amount of adult immune serum was added.

In a group of 9 infants, the degree of phagocytosis was definitely increased as a result of intravenous blood transfusions.

In 5 infants who received 20 cc. of adult immune blood, and in 13 infants who received 10 cc. of hyper-immune human serum intramuscularly, there was definite but less striking increase in phagocytosis.

Placental extract injected intramuscularly into 3 infants in dosages of 10 cc. produced a definite increase in the phagocytic power of the blood. A similar result was obtained when 1 infant was injected with 20 cc. of immune rabbit serum.

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# THE BISULPHITE BINDING POWER OF THE BLOOD IN HEALTH AND IN DISEASE, WITH SPECIAL REFERENCE TO VITAMIN B<sub>1</sub> DEFICIENCY<sup>1</sup>

By F. H. L. TAYLOR, SOMA WEISS AND ROBERT W. WILKINS

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston)

(Received for publication May 4, 1937)

There is increasing evidence that the biological function of vitamin B<sub>1</sub> is concerned with oxidative processes in the living organism. In studies of oxygen utilization by manometric methods, Peters and his coworkers (1, 2) have shown that there is increased oxygen uptake by deficient brain and kidney tissues *in vitro* following the addition of vitamin B<sub>1</sub>. In the same laboratory Thompson and Johnson (3, 4) found increased amounts of carbonyl compounds in the blood of B<sub>1</sub> avitaminotic pigeons and rats. Clinical confirmation has recently been given these experimental findings by Platt and Lu (5), who reported marked increases in the carbonyl compounds in the blood of patients with acute beriberi in the Orient.

These authors measured the carbonyl compounds by means of the bisulphite binding power of the blood. Pyruvic acid has received most attention, chiefly through the emphasis given to it by Peters et al. (2). Thompson and Johnson concluded that the major portion of the increase in bisulphite binding substances (B.B.S.) in the blood of pigeons and rats severely deficient in vitamin B<sub>1</sub> was due to pyruvic acid. On the other hand, Sherman and Elvehjem (6) found no increase in the B.B.S. in the blood of chickens deficient in vitamin B<sub>1</sub>, but did find that these birds, in contrast to normal birds, showed a sharp rise in blood B.B.S. after the intravenous injection of sodium pyruvate. Platt and Lu in a recent report (7) state that the increases in B.B.S. in the blood of fulminating cases of beriberi are due mainly to pyruvic acid.

For 2 years an investigation has been in progress in this laboratory on patients with symptoms and signs of vitamin B<sub>1</sub> deficiency closely resembling those seen in oriental "wet" beriberi (8,

9). The present study of the B.B.S. in the blood was undertaken in order (1) to throw further light on the relationship of these cases of vitamin B<sub>1</sub> deficiency to those reported in other parts of the world; (2) to relate the B.B.S. in the blood to clinical and laboratory findings in various medical diseases in order to determine the factors which influence the B.B.S.; and (3) to ascertain the diagnostic and prognostic value of measurements of the B.B.S. in the blood.

## SELECTION OF CASES

The present communication reports the findings in a group of 174 persons, consisting of 30 normal subjects and 144 patients. The control subjects were members of the hospital staff and were free from any evidence of disturbed metabolism, infection or vitamin deficiency. The patients were chosen from the medical wards of this hospital and were predominantly persons with metabolic disturbances, including vitamin B<sub>1</sub> deficiency. An additional group of patients was selected in order to determine the rôle of certain diseases which frequently complicate vitamin B<sub>1</sub> deficiency, such as alcoholism.

The distribution of the patients is shown in Table I. In assigning a patient to the group with

TABLE I  
Distribution of cases

Principal diagnosis	Number of cases
Organic heart disease . . . . .	35
Probable vitamin B <sub>1</sub> deficiency . . . . .	28
Infectious diseases . . . . .	17
Diabetes mellitus . . . . .	13
Nephritis . . . . .	10
Alcoholism . . . . .	8
Liver disease . . . . .	8
Pregnancy . . . . .	6
Neoplasms . . . . .	5
Miscellaneous diseases . . . . .	14

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billion organisms. The donor was bled two weeks after the final injection, the serum harvested and preserved by the addition of 0.25 per cent tricresol. Kendrick (5) has reported excellent prophylactic results from the use of similar serum in exposed infants. The increase in phagocytosis brought about by this serum is not as great as that produced by transfusion, but consists chiefly in a shift from "none to slight" to "definite."

The effect of injecting 10 cc. of placental extract was studied in 3 infants and, as shown in Table V, a definite increase in the phagocytic

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*The effect of intramuscular injection of 10 cc. of placental extract upon phagocytosis of H. pertussis*

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So...	3	2	13	10	0	8	17
Ka...	9	1	24	0	0	15	10
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Liver disease.....	8
Pregnancy.....	6
Neoplasms.....	5
Miscellaneous diseases.....	14

<sup>1</sup> This investigation was aided in part by a grant from the Josiah Macy, Jr. Foundation.

probable vitamin B<sub>1</sub> deficiency, the presence of one or more of the following clinical criteria was required in addition to a history of a diet grossly deficient in vitamin B<sub>1</sub> and improvement after vitamin B<sub>1</sub> therapy: peripheral polyneuritis, pellagra, Korsakoff type of psychosis, congestive failure of the circulation without any other adequate explanation than vitamin B<sub>1</sub> deficiency, or polyavitaminosis with malnutrition. It is appreciated that such a classification is not rigidly accurate as the criteria used depend largely on the interpretation of history and physical findings. Furthermore, it is recognized that the patients in this group usually suffered from a deficiency not only of B<sub>1</sub> but also of the other fractions of the vitamin B complex as well as a deficiency of other vitamins. Because of the frequent association in this hospital of chronic alcoholism with avitaminosis, a relatively large number of alcoholic patients were included.

Certain patients were treated during the period of observation with the specific therapy required for their condition. In such cases repeated observations were usually made both before treatment (Part A of the tables) and after treatment (Part B). Attention is called to these cases by means of an asterisk. Because of lack of space only one series of observations before and one series after treatment are presented.

#### METHODS

Blood was taken from patients fasting and at rest and delivered into bottles containing dry potassium oxalate. Duplicate samples were analyzed without delay.

*Bisulphite binding power.* Slight modifications were made in the method of Clift and Cook (10) in order to adapt it to whole blood determinations. Five ml. of oxalated blood were precipitated with 20 ml. of 10 per cent trichloroacetic acid, allowed to stand 30 minutes and centrifuged. The clear supernatant fluid was removed from the precipitated protein. Determinations were made immediately, although it was found that the protein-free filtrate might be kept in the ice chest overnight without change in bisulphite binding power.

Five ml. aliquots of the supernatant fluid were adjusted to pH 2 by the addition of 1.5 ml. of normal sodium hydroxide, and allowed to react for at least 15 minutes with 0.2 ml. of saturated sodium bisulphite solution. Accurate control of the pH is not essential, but the alkalinity should not be permitted to rise above pH 4.

The solution was then diluted with 25 ml. of distilled water, 2 ml. of freshly prepared 1 per cent starch solu-

tion added, and the excess of bisulphite titrated out, first using normal and N/10 iodine solutions and finally adjusting the end point with N/200 iodine and N/100 thiosulphate solutions. The volume at the end of this part of the procedure should be approximately 65 ml. The preliminary dilution prevents the formation of a yellowish compound, probably an iodate-iodine combination, which may cause high results. At pH 2 the reactions of iodine and thiosulphate solutions proceed more slowly than when the solutions are acid with 6 N hydrochloric acid, as is usual in iodimetry. For this reason the titration of excess bisulphite must be done slowly; otherwise some thiosulphate remains unchanged and increases the bound bisulphite titer.

The bound bisulphite is then released by the addition of 2 grams of solid disodium phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ), mixing for 5 minutes by rotation to avoid aeration. The released bisulphite is then titrated with N/200 iodine solution. The end point chosen is that at which a definite blue color persists for 30 seconds. Blanks are run on the reagents and these may be used as reference solutions for the end point. The bisulphite binding power is expressed as milligrams of pyruvic acid, using the equation: 1 ml. N/200 iodine solution = 0.22 mgm. pyruvic acid.

The N/200 iodine solution should be standardized once a week, and when many determinations are being run the burette should be refilled with fresh iodine solution every hour.

Blood sugar and nonprotein nitrogen were determined by the method of Folin (11), plasma proteins by a modification of the Howe procedure (12, 13) and carbon dioxide capacity by the method of Van Slyke (14). Urines were examined for acetone and diacetic acid by the routine clinical tests, and in addition for pyruvic acid by a modification (15) of the Simon-Piaux method.

#### RESULTS

The bisulphite binding power of the blood in the normal subjects ranged from 3.7 to 5.8 mgm. per 100 ml. of whole blood, with an average of 4.7 mgm. It has been arbitrarily decided to consider all values above 6 mgm. per 100 ml. as increased, and values below 3.5 mgm. per 100 ml. as decreased.

Table II gives the diseases in which an increase in the B.B.S. in the blood was found, and the incidence of increased B.B.S. in each condition. In addition to these diseases, the bisulphite binding power of the blood was found increased in seven other conditions, with only one example of each. This group included chronic vomiting, therapeutically induced ketosis in epilepsy, bichloride poisoning with nitrogen retention, acute hepatitis

TABLE II  
*Diseases with increased B.B.S. in the blood*

Diagnosis	Number of cases	Number with elevated B.B.S.	B.B.S. (as pyruvic acid)		
			Minimum	Maximum	Average
			<i>mgm. per 100 ml.</i>	<i>mgm. per 100 ml.</i>	<i>mgm. per 100 ml.</i>
Organic heart disease (decompensated).....	19	10	4.2	8.2	6.2
Vitamin B <sub>1</sub> deficiency (untreated).....	23	18	4.6	51.9	12.1
Infectious diseases (febrile).....	9	6	3.7	9.2	6.3
Diabetes with acidosis.....	7	7	6.8	109.2	38.6
Nephrosclerosis.....	4	2	3.7	7.8	5.7

with fever, Hodgkin's disease complicated by chronic nephrosclerosis, diabetes mellitus complicated by arteriosclerosis, pericarditis and nephritis, and pancreatitis complicated by vomiting, myocarditis and nephritis.

Table III presents the findings in conditions in which no increase in the B.B.S. in the blood oc-

*The relationship of the bisulphite binding power of the blood to vitamin B<sub>1</sub> deficiency and to alcoholism*

Table IV-A presents the data on 23 cases of probable vitamin B<sub>1</sub> deficiency observed before treatment. The cases are arranged in order of severity as determined by clinical observation.

TABLE III  
*Conditions with no increase in B.B.S. in the blood*

Diagnosis	Number of cases	B.B.S. (as pyruvic acid)		
		Minimum	Maximum	Average
		<i>mgm. per 100 ml.</i>	<i>mgm. per 100 ml.</i>	<i>mgm. per 100 ml.</i>
No disease.....	30	3.7	5.8	4.7
Organic heart disease (compensated) *.....	19	3.0	5.7	4.2
Vitamin B <sub>1</sub> deficiency (treated) *.....	14	2.9	5.8	4.5
Infectious diseases (afebrile) *.....	12	3.7	5.2	4.4
Diabetes mellitus (regulated) *.....	10	3.1	5.8	4.8
Liver disease.....	8	2.5	4.8	4.1
Pregnancy.....	6	3.4	4.2	3.7
Glomerulonephritis.....	5	4.9	5.8	5.3
Neoplasms.....	5	4.4	5.5	5.0
Acute alcoholism.....	4	4.0	5.3	4.7
Chronic alcoholism.....	4	4.7	5.0	4.9
Severe anemia.....	2	3.0	3.4	3.2
Senility.....	2	3.6	4.8	4.2

\* This group includes certain cases appearing in Table II which, subsequent to treatment, showed no elevation of B.B.S. in the blood.

curred. In addition, the B.B.S. was not increased in 1 case each of hyperthyroidism, starvation, nephrosis, and hypoproteinemia associated with chronic colitis.

From such a selection of cases no conclusions can be drawn concerning the incidence of increased B.B.S. in medical diseases. The frequent association of certain of the diseases studied with an increase in the B.B.S. in the blood, nevertheless, requires further scrutiny.

Cases 1 to 8, inclusive, were regarded as severe, if not "fulminating," and were characterized by marked congestive failure of the circulation with massive edema, as well as by varying degrees of peripheral neuritis and psychosis. It will be seen that there was no uniform relationship between the clinical severity of the disease and the amount of the B.B.S. in the blood. Nevertheless 17, or 74 per cent, of the untreated group had an increase in the B.B.S. This is in striking contrast

TABLE IV  
Cases with vitamin B<sub>1</sub> deficiency  
(Arranged in order of severity of symptoms)

Case number	Sex	Age	Time of test (day in hospital)	B.B.S. (as pyruvic acid)	Non-protein nitrogen	CO <sub>2</sub> capacity	Blood sugar	Urine analysis				Course
								Acetone	Di-acetic	Py-ruvic	Sugar	
		years		mgm. per 100 ml.	mgm. per 100 ml.	volumes per cent	mgm. per 100 ml.					
A. UNTREATED CASES												
1	M.	43	1	13.9	80	47	103	0	0	Trace	0	Died
2	M.	49	4	9.6	82	49	132	0	0	0	0	Improved
3	M.	35	3	14.6	30†	45		++	0	0	0	Improved
4	M.	60	5	8.3	32†	75	122	+	0	+	0	Improved
5	F.	52	16	7.0	28†	60		0	0	0	0	Died
6	M.	69	4	2.8	26†			0	0		0	Improved
7	M.	42	6	4.6	32			0	0		0	Died
8	M.	69	3	7.3	23			0	0		0	Improved
9	F.	55	3	8.4	38†			0	0		+	Died
10	F.	38	3	7.1	32			0	0		+	Improved
11	M.	43	7	4.0	33	63	93	0	0	0	0	Improved
12	M.	48	4	6.3	39†	61		0	0	0	0	Improved
13	M.	42	5	8.1	44			0			0	Improved
14	M.	51	4	5.8	43			0			0	Improved
15	M.	60	1	13.7		57	109	++	0	±	Trace	Improved
16	F.	29	3	51.9	19	59	80†	±	0	+	0	Improved
17	M.	47	2	50.8	45		232	0			0	Improved
18	M.	36	3	7.9	36	61	99	0	0	0	0	Improved
19	M.	46	2	15.1	37	70	124	++++	0	+	+	Improved
20	M.	36	2	11.0	23†	65		++++	0	0	Trace	Improved
21	M.	37	2	7.3	22	52	153	++++	0	+	Trace	Improved
22	M.	56	2	5.1	50			0			0	Improved
23	M.	41	6	4.9	25							Improved
B. TREATED CASES												
												Treatment
2*	M.	49	6	5.6	50†	60	93	0	0	0	0	Crystalline vitamin B <sub>1</sub> , 2 days
3*	M.	35	5	5.8	27†	61		±	0	0	0	Crystalline vitamin B <sub>1</sub> , 2 days
4*	M.	60	7	4.7	37†	60		±	0	0	0	Crystalline vitamin B <sub>1</sub> , 2 days
6*	M.	69	5	3.2	29							Crystalline vitamin B <sub>1</sub> , 1 day
8*	M.	69	23	3.5	33			0	0		0	High vitamin diet, 2 weeks
11*	M.	43	16	5.0		65	87	0	0	0	0	Crystalline vitamin B <sub>1</sub> , 4 days
12*	M.	48	5	4.1		58	93	0	0	0	0	Crystalline vitamin B <sub>1</sub> , 1 day
16*	F.	29	6	3.9	28	65		0	0	0	Trace	Extract vitamin B <sub>1</sub> , 3 days
19*	M.	46	11	3.8	33	59	92	0	0	0	0	Crystalline vitamin B <sub>1</sub> , 5 days
24	F.	29	60	2.9	31							Extract vitamin B <sub>1</sub> , 1 month
25	M.	45	6	5.0	37							Extract vitamin B <sub>1</sub> , 1 day
26	F.	29	7	4.8	33			0	0	0	0	High vitamin diet, 3 days
27	M.	45	7	4.8	34	54	97	0	0	0	0	Extract vitamin B <sub>1</sub> , 2 days
28	M.	52	3	5.0	12†	61		0	0	0	0	High vitamin diet, 3 months

\* These cases appear in section A of this table. The other cases received treatment before any observations were made.

† Determination on plasma.

to a group of the same and similar patients who after vitamin B<sub>1</sub> therapy (Table IV-B) had consistently normal B.B.S. in the blood.

Because all but one of the deficient patients (Case 9) were also alcoholic, it was necessary to determine the effect, if any, of alcohol *per se* on the B.B.S. A control group of 8 cases was

selected, 4 with acute alcoholism (in coma) and 4 with chronic alcoholism without signs or symptoms of vitamin deficiency and without manifestations of acute alcoholic intoxication (Table V). In no instance was there an increase in the B.B.S. in the blood. This finding and the fact that the cases of chronic alcoholism and vitamin B<sub>1</sub> defi-

ciency showed a prompt return of the B.B.S. to normal after crystalline vitamin B<sub>1</sub> therapy (Table IV-B) indicate that the increase in the B.B.S. in these cases is related to the vitamin deficiency and not directly to the chemical or physiological effects of alcohol.

The increase in the B.B.S. in the blood was not the only evidence of abnormal metabolism in the

cases of vitamin B<sub>1</sub> deficiency. The fasting blood sugar was increased in 4 cases (Table IV-A), but returned to normal after treatment with crystalline vitamin B<sub>1</sub> or adequate diet. Moreover, sugar, acetone, diacetic acid and pyruvic acid were found in the urine of several patients before treatment, but were rarely present after vitamin therapy.

TABLE V  
*Cases with alcoholism without vitamin B<sub>1</sub> deficiency*

Case number	Sex	Age	Time of test (day in hospital)	Type	B.B.S. (as pyruvic acid)	Nonprotein nitrogen
		years			mgm. per 100 ml.	mgm. per 100 ml.
29	M.	32	1	Acute	4.2	
30	M.	32	1	Acute	5.3	
31	M.	30	1	Acute	5.2	
32	M.	35	1	Acute	4.0	
33	M.	45	3	Chronic	4.7	48
34	M.	38	3	Chronic	4.8	38
35	M.	51	3	Chronic	4.9	32
36	M.	67	8	Chronic	5.0	41

TABLE VI  
*Cases with diabetes mellitus*  
(Arranged in order of severity of symptoms)

Case number	Sex	Age	Time of test (day in hospital)	B.B.S. (as pyruvic acid)	Non-protein nitrogen	CO <sub>2</sub> capacity	Blood sugar	Urine analysis			
								Acetone	Diacetic	Pyruvic	Sugar
		years		mgm. per 100 ml.	mgm. per 100 ml.	volumes per cent	mgm. per 100 ml.				

#### A. UNREGULATED CASES

36	F.	60	1	109.2	64	22	471	++++	Trace	+	++
37	F.	14	1	63.9		29	187	++++	Trace	0	+
38	F.	17	2	37.7	77	23	293	+	0		+++
39	M.	34	1	33.2	24	23	292	++++	0		++++
40	F.	50	1	11.0	46	60	274	++	0	+	+
41	F.	55	1	8.4	46		305	+++	Trace	+	+++
42	F.	65	2	6.8	46		286	0			+

#### B. REGULATED CASES

37*	F.	14	35	4.3	30	62	147	0	0	0	0
38*	F.	17	13	4.9		69	48	0	0	0	0
39*	M.	34	16	5.0	29		203	0	0	0	+
41*	F.	55	7	5.2		64	199	0	0	0	0
43	F.	53	34	5.1		62		Trace	0	0	+
44	F.	56	20	5.6	31		175	0	0		+
45	F.	52	2	5.3	31	61	212	0	0	0	+
46	M.	14	12	4.4	39		164	0	0	0	+
47	F.	39	5	3.1	30		164	0			+
48	M.	51	2	5.8	16	61	242	Trace	0	0	+

\* These cases appear in section A of this table. Under treatment, they became regulated.

*The bisulphite binding power of the blood in conditions other than vitamin B<sub>1</sub> deficiency*

*Diabetes mellitus.* Table VI presents the data on: (A) "unregulated" diabetic patients and (B) "regulated" diabetic patients. Cases in the "unregulated" group showed elevated sugar and lowered carbon dioxide capacity of the blood, and

sugar, acetone and diacetic acid in the urine. Cases in the "regulated" group at times had elevation of the blood sugar, but it was not accompanied by acidosis or ketosis.

Certain points emerge from a comparison of the two groups. In the unregulated patients there was an increase in the B.B.S. in the blood, which

TABLE VII  
*Cases with organic heart disease*  
(Arranged in order of severity of symptoms)

Case number	Sex	Age	Type of heart disease†	Time of test (day in hospital)	B.B.S. (as pyruvic acid)	Non-protein nitrogen	Urine analysis			Course
							Acetone	Di-acetic	Pyruvic	
		years			mgm. per 100 ml.	mgm. per 100 ml.				
A. DECOMPENSATED CASES										
49	M.	65	C. T.	3	6.6	50				Died
50	M.	68	C. T.	5	8.2	211				Died
51	F.	53	R.	3	7.9	35	0			Died
52	M.	60	L.	49	4.2	40	0	0	0	Died
53	M.	59	R.	7	6.6	49	0	0		Died
54	M.	66	C. T.	5	5.9	48	0			Died
55	F.	42	R.	3	5.6	88	0	0		Died
56	M.	48	A. C.	28	6.3	39				Died
57	M.	57	H. A.	2	7.5	43	0			Improved
58	M.	63	A.	2	5.8	43				Improved
59	M.	55	H.	2	5.6	48	0			Improved
60	F.	69	H. A.	3	5.0	32				Improved
61	M.	43	R.	24	7.2	35	0	0	0	Improved
62	M.	56	H. C.	50	5.0	27				Improved
63	M.	56	R.	6	7.5	37	0			Improved
64	M.	63	C. T.	2	5.7	87				Improved
65	M.	54	C.	19	4.2	37				Improved
66	F.	65	H. A.	18	6.9	80	0			Improved
67	M.	52	C.	4	6.5	38	0			Improved
B. COMPENSATED CASES										
68	F.	36	R.	6	3.4	36	0			Improved
69	M.	62	A. C.	21	3.1	37				Died
70	F.	71	H. A.	4	5.7	50	0			Improved
71	M.	73	H. A.	5	3.0	62	0	0		Improved
72	M.	40	R.	20	4.0	32	0			Improved
73	M.	75	A.	4	3.7	39				Improved
57*	M.	57	H. A.	30	4.5	33	0			Improved
63*	M.	56	R.	30	3.7	38	0	0	0	Improved
74	M.	36	C.	13	3.3	37				Improved
75	M.	40	H.	27	4.2	42	0			Improved
76	M.	67	A.	4	3.2	37	0	0		Improved
77	M.	54	A.	12	3.6	20	0			Improved
78	M.	40	R.	47	5.5					Improved
79	M.	59	H.	3	5.4	52	0	0		Improved
80	M.	68	H. A.	5	3.4	41	0			Improved
81	M.	72	H. A.	2	5.1		0			Improved
82	M.	77	A.	30	5.3	44				Improved
67*	M.	52	C.	30	4.8	38	0			Improved
83	M.	19	R.	83	4.7					Improved

\* These cases appear in section A of this table. Under therapy they became compensated.

† A. = Arteriosclerotic. C. = Coronary. H. = Hypertensive. R. = Rheumatic. L. = Luetic. C. T. = Coronary thrombosis.



in some cases was extreme. The degree of elevation was related more directly to the "clinical" condition of the patient than to the acidosis as measured by the carbon dioxide capacity, or even to the ketosis as measured by ketone bodies in the urine. It bore no relation whatever to the level of the blood sugar. After treatment with insulin and fluids, the B.B.S. rapidly returned to normal and again closely paralleled the clinical appearance of the patient. The level of the B.B.S. in the blood is an accurate index of the severity of the metabolic disturbances and of the success of therapy in diabetic patients.

Tests for pyruvic acid were positive in the urines of several of the unregulated diabetic patients, a finding also recently observed by others (16). The rôle of pyruvic acid in the acidosis and in the elevation of the B.B.S. in diabetes awaits further observations.

*Chronic organic heart disease.* The association of cardiac disturbances with vitamin B<sub>1</sub> deficiency has been established in animals (17, 18). It is also generally recognized that cardiovascular dysfunctions occur in beriberi in the Orient (19, 20) and in vitamin "B" deficiencies in the United States (8, 9, 21). In order to determine what effect heart disease, with or without congestive failure, has on the B.B.S. in the blood, data were obtained on a control group of 35 patients with chronic organic heart disease without clinical evidence of vitamin deficiency. The data on this group are presented in Table VII: (A) "decompensated" patients with marked signs of congestive failure of the circulation; and (B) "compensated" patients with slight or no evidence of congestive failure. In the table the cases are arranged in order of clinical severity of decompensation, as determined by the amount of edema, venous distention, dyspnea, orthopnea and signs of pulmonary congestion that they manifested. It will be seen that in patients with organic heart disease there is a general correlation between the clinical severity of congestive failure and the elevation in the B.B.S. in the blood. The incidence of increased B.B.S. even in the decompensated group (52 per cent) was not so high as in patients with vitamin B<sub>1</sub> deficiency (74 per cent), nor was the degree of elevation so great (average B.B.S. 6.2 mgm. as compared with 12 mgm. per 100 ml.). Thus congestive failure alone, without de-

monstrable vitamin deficiency, may be associated with increased B.B.S. in the blood. Congestive circulatory failure may therefore contribute to the elevation of the B.B.S. in certain cases of vitamin B<sub>1</sub> deficiency with edema ("wet" beriberi). Following improvement under therapy with rest, digitalis and diuretics, the B.B.S. in the blood of cardiac patients returned to normal (Table VII-B).

*Infections.* The relationship of infection to vitamin deficiency and to the onset of clinical avitaminosis has often been commented on in the literature. The frequent occurrence of febrile episodes during the course of beriberi and pellagra and the aggravation of the symptoms of these diseases by infections have led many observers to accept the "infectious" theory of their etiology. Observations were therefore made on 17 patients with various infectious diseases in order to determine the relation of infection, and particularly of fever, to the B.B.S. in the blood. Table VIII presents the data on: (A) cases with febrile infections and (B) cases with infections which at the time of the measurements were afebrile. In none of the patients was there clinical evidence of vitamin deficiency. The cases are arranged in order of severity of the infection as judged from the height of the fever and the pulse rate, and from the degree of prostration of the patient.

There is a fairly close correlation between the elevation of the B.B.S. and the severity of the infection. That fever *per se* is not responsible for the increased B.B.S. is shown by Case 89, in which there was normal B.B.S. but high fever due to severe tuberculosis. Dehydration with reduced kidney function, and ketosis as evidenced by acetoneuria, undoubtedly play contributing rôles. It is also possible that anoxemia, such as occurs in lobar pneumonia, may be a factor in the metabolic disturbances which are associated with increased B.B.S. in the blood of some febrile patients. With recovery the B.B.S. in these patients returned to normal (Cases 84, 85, 86, 87). Afebrile infections were not associated with an increase in the B.B.S.

*Nephritis.* Five patients with glomerulonephritis, 4 with nephrosclerosis and 1 with nephrosis were studied. Among the patients with glomerulonephritis the nonprotein nitrogen varied from 35 mgm. per 100 ml. to 162 mgm. with no elevation

TABLE VIII  
*Cases with infections*  
 (Arranged in order of severity of symptoms)

Case number	Sex	Age	Diagnosis	Time of test (day in hospital)	B.B.S. (as pyruvic acid)	Non-protein nitrogen	Urine analysis		
							Acetone	Di-acetic	Py-ruvic
		years			mgm. per 100 ml.	mgm. per 100 ml.			
A. FEBRILE CASES									
84	F.	14	Lobar pneumonia	4	8.0	48	++	0	0
85	M.	40	Lobar pneumonia	2	8.1	43			
86	M.	38	Lobar pneumonia	3	6.1	18	++		
87	M.	46	Lobar pneumonia	5	9.2				
88	M.	24	Extensive tuberculosis	51	6.4				
89	F.	28	Pulmonary tuberculosis	6	3.7				
90	M.	56	Malaria	8	3.9	32	0		
91	F.	16	Minimal tuberculosis	7	4.3	37	0		
92	M.	16	Acute respiratory infection	2	6.4		Trace		
B. AFEBRILE CASES									
93	M.	32	Acute respiratory infection	5	3.9	36	0	0	0
94	M.	28	Prostatitis; gonococcal arthritis	12	5.2	34	0	0	0
95	M.	45	Meningitis	30	4.4	25			
96	M.	24	Primary syphilis	11	4.2		0	0	0
97	M.	27	Primary syphilis	14	3.7	25	0	0	0
98	M.	25	Primary syphilis	15	4.0	34	0	0	0
99	M.	32	Primary syphilis	9	5.2	33	0	0	0
100	M.	32	Rheumatic fever	60	4.1				
84*	F.	14	Convalescent; lobar pneumonia	8	3.9		0	0	0
85*	M.	40	Convalescent; lobar pneumonia	6	4.6	42			
86*	M.	38	Convalescent; lobar pneumonia	7	4.0	30			
87*	M.	46	Convalescent; lobar pneumonia	8	5.1	36			

\* These cases appear also in section A of this table.

of the bisulphite binding power. Two of the nephrosclerotic patients with nonprotein nitrogen of 96 and 78 mgm. per 100 ml. had values for B.B.S. in the blood of 6.1 and 7.8 mgm. per 100 ml., respectively. In the second case the carbon dioxide combining power was 47 volumes per cent. These two groups of patients show that there is no direct relationship between the non-protein nitrogen and the bisulphite binding power of the blood. The patient with nephrosis showed the lowest B.B.S. recorded: 1.7 mgm. per 100 ml.

*Jaundice and liver disease.* Eight patients with various forms of jaundice and liver disease were studied. These included 5 cases of cirrhosis, 1 case each of catarrhal jaundice, of arsphenamine hepatitis and of cirrhosis complicated by diabetes mellitus with a blood sugar of 168 mgm. per 100 ml. The B.B.S. was not elevated in any of these patients.

*Pregnancy.* Six patients were studied in the

third trimester of pregnancy. One patient had hypoproteinemia, 2 had essential hypertension and 3 had so-called "toxemia" of pregnancy. The carbon dioxide combining power varied between 50 and 57 volumes per cent. In no instance was there an elevated B.B.S.

*Neoplasms.* Five patients suffering from various forms of malignant disease, including 1 case of Hodgkin's disease, were observed. In no case was the B.B.S. elevated.

*Miscellaneous.* No elevation of the B.B.S. was found in 2 cases of senility with arteriosclerosis or in 1 case each of secondary anemia, pernicious anemia, hypoproteinemia with malnutrition, and hyperthyroidism.

One patient suffering from epilepsy when placed on a therapeutic ketogenic diet showed an increase in B.B.S. to 21 mgm. per 100 ml. In this patient elevation of the B.B.S. occurred without any lowering of the carbon dioxide capacity

of the blood. One patient with pernicious vomiting and acidosis showed an elevation of the B.B.S. to 13 mgm. per 100 ml.

*Cases with multiple diseases.* There were 7 cases in which a single diagnosis could not be made. In 5 of these the B.B.S. was elevated above normal limits. They were cases in which any one of the presenting diseases may be associated with an elevation of the B.B.S. The data on these patients are given in Table IX.

*The relationship of the B.B.S. to certain other constituents of the blood*

*Glucose.* Under the conditions of the method outlined above, bisulphite is not bound by glucose. This was established by the following experimental considerations: (1) The addition of glucose to blood *in vitro* caused no change in the bisulphite binding power. (2) The intravenous injection of 100 cc. of 50 per cent glucose solution caused no change in the bisulphite binding

TABLE IX  
*Cases with multiple diseases*

Case number	Sex	Age years	Diagnosis	Time of test (day in hospital)	B.B.S. (as pyruvic acid) mgm. per 100 ml.	Non-protein nitrogen mgm. per 100 ml.	Urine analysis		Additional findings
							Acetone	Di-acetic	
101	M.	46	Chronic alcoholic cirrhosis;	3					
102	F.	22	acute hepatitis; fever		9.5	24	0	0	
103	F.	63	Congenital syphilis; pregnancy; pyelitis; jaundice; fever	30	2.6	28	0	0	
104	F.	28	Arteriosclerosis; diabetes; nephritis; heart failure	23	11.5	78	0		
105	M.	60	Pancreatitis; nephritis; myocarditis; vomiting	38	8.5	42	0		Blood sugar 258
106	M.	55	Bichloride poisoning; uremia	14	7.4	176	0	0	Carbon dioxide 29
107	M.	26	Hodgkin's disease; coronary heart disease; chronic nephritis	11	8.4	56			
			Chronic colitis; hypoproteinemia	3	3.6	26	0	0	Carbon dioxide 50

*Diseases associated with decreased B.B.S. in the blood*

Eighteen patients showed B.B.S. ranging from 1.9 to 3.5 mgm. per 100 ml. Serum protein analyses were made on 8 of these patients. All 8 had hypoproteinemia. Low B.B.S. was found in 5 cases with heart disease of arteriosclerotic or hypertensive origin, in 4 cases of pregnancy, in 2 cases of cirrhosis, in 2 cases of nutritional deficiency, in 1 patient with chronic passive congestion of the circulation due to tricuspid stenosis, and in 1 case each of nephrosis, pernicious anemia, and severe secondary anemia. The most common finding associated with a lowering of the B.B.S. in this study was the reduction in the serum proteins.

power, although it greatly increased the blood sugar. (3) In diabetic patients with elevated blood sugar but without acidosis the bisulphite binding power of the blood was not increased. There was no relationship between the B.B.S. and the glucose in the blood.

*Nonprotein nitrogen.* Patients suffering from glomerulonephritis with increases in the non-protein nitrogen showed no increase in the B.B.S. In clinical "uremia," however, an increase in the nonprotein nitrogen was often accompanied by an increase in the B.B.S. With amelioration of the uremic condition the elevated B.B.S. returned to normal.

*Carbon dioxide capacity of the blood.* There appears to be no direct relationship between the B.B.S. and the carbon dioxide capacity of the

TABLE VIII  
Cases with infections  
(Arranged in order of severity of symptoms)

Case number	Sex	Age	Diagnosis	Time of test (day in hospital)	B.B.S. (as pyruvic acid)	Non-protein nitrogen	Urine analysis		
							Acetone	Di-acetic	Py-ruvic
		years			mgm. per 100 ml.	mgm. per 100 ml.			
A. FEBRILE CASES									
84	F.	14	Lobar pneumonia	4	8.0	48	++	0	0
85	M.	40	Lobar pneumonia	2	8.1	43			
86	M.	38	Lobar pneumonia	3	6.1	18	++		
87	M.	46	Lobar pneumonia	5	9.2				
88	M.	24	Extensive tuberculosis	51	6.4				
89	F.	28	Pulmonary tuberculosis	6	3.7				
90	M.	56	Malaria	8	3.9	32	0		
91	F.	16	Minimal tuberculosis	7	4.3	37	0		
92	M.	16	Acute respiratory infection	2	6.4		Trace		
B. AFEBRILE CASES									
93	M.	32	Acute respiratory infection	5	3.9	36	0	0	0
94	M.	28	Prostatitis; gonococcal arthritis	12	5.2	34	0	0	0
95	M.	45	Meningitis	30	4.4	25			
96	M.	24	Primary syphilis	11	4.2		0	0	0
97	M.	27	Primary syphilis	14	3.7	25	0	0	0
98	M.	25	Primary syphilis	15	4.0	34	0	0	0
99	M.	32	Primary syphilis	9	5.2	33	0	0	0
100	M.	32	Rheumatic fever	60	4.1				
84*	F.	14	Convalescent; lobar pneumonia	8	3.9		0	0	0
85*	M.	40	Convalescent; lobar pneumonia	6	4.6	42			
86*	M.	38	Convalescent; lobar pneumonia	7	4.0	30			
87*	M.	46	Convalescent; lobar pneumonia	8	5.1	36			

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of the bisulphite binding power. Two of the nephrosclerotic patients with nonprotein nitrogen of 96 and 78 mgm. per 100 ml. had values for B.B.S. in the blood of 6.1 and 7.8 mgm. per 100 ml., respectively. In the second case the carbon dioxide combining power was 47 volumes per cent. These two groups of patients show that there is no direct relationship between the non-protein nitrogen and the bisulphite binding power of the blood. The patient with nephrosis showed the lowest B.B.S. recorded: 1.7 mgm. per 100 ml.

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105	M.	60	Bichloride poisoning; uremia	14	7.4	176	0	0	
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*Carbon dioxide capacity of the blood.* There appears to be no direct relationship between the B.B.S. and the carbon dioxide capacity of the

blood. A simple lowering of the carbon dioxide capacity in several patients by means of ammonium chloride therapy even to the point of clinical acidosis produced no change in the B.B.S. There is, however, a distinct relationship between the B.B.S. and certain underlying factors which may result in a lowering of the carbon dioxide capacity. Patients with diabetic acidosis, for example, in whom the decreased carbon dioxide capacity was due primarily to ketosis showed exceedingly high B.B.S. in the blood. On the other hand, a patient on a ketogenic régime for epilepsy had a high B.B.S. but a normal carbon dioxide capacity.

*Pyruvic acid.* The relationship of the B.B.S. to pyruvic acid requires further investigation. As judged from the amount of this material found in the urine and from preliminary work on direct measurement of the substance in the blood, pyruvic acid alone cannot account for the increased B.B.S. in the blood of patients with vitamin B<sub>1</sub> deficiency or the other diseases observed with an elevation of the B.B.S.

*Drugs.* Many of the patients under observation were of necessity given various sedative drugs. Frequently, the medication was administered on admission to the hospital. This was particularly true of patients with acute or chronic alcoholism. Chief interest centered in such drugs as chloral hydrate and paraldehyde, which have free carbonyl groups. In 3 instances it was found that the administration of large doses of these drugs was followed by a slight rise in the B.B.S. Several patients, however, after equally large amounts of the same sedatives showed normal B.B.S. Drugs other than chloral hydrate and paraldehyde showed no effect on the B.B.S.

#### *The B.B.S. and rest*

In some cases the routine treatment and diet alone, with no specific therapy, reduced the B.B.S. in the blood. This was true not only in the vitamin B<sub>1</sub> deficient group but also in all types of cases in which an increase in B.B.S. occurred. The lowering of the B.B.S. in these cases was ascribed primarily to the effects of bed rest and decrease in total metabolism, but undoubtedly the administration of fluids and probably other factors played a rôle. For this reason the length of time

after admission that the test was done often had a bearing on the height of the B.B.S.

#### DISCUSSION

The observations presented indicate that elevation of B.B.S. in the blood is not specific in vitamin B<sub>1</sub> deficiency, since it occurs in other diseases, such as diabetes mellitus, certain infectious diseases and severe congestive failure of the circulation caused by organic heart disease. In these diseases the total B.B.S. cannot always be accounted for by the presence of acetone and diacetic acid. In certain cases of vitamin B<sub>1</sub> deficiency high B.B.S. in the blood was observed without acetone, diacetic or pyruvic acid in the urine, suggesting that other carbonyl compounds had accumulated in the blood. It is of significance that there were instances both of polyneuritis and of cardiac insufficiency of nutritional origin with low vitamin B<sub>1</sub> intake in which the B.B.S. was normal. Thus a simple correlation was not found between the level of B.B.S., on the one hand, and the severity of the nutritional polyneuritis and cardiovascular dysfunction, on the other. These findings are not entirely in agreement with those of Platt and Lu, but they are in accord with the studies of de Jong (22) on pigeons with vitamin B<sub>1</sub> deficiency. De Jong did not find a simple correlation between the manifestations of vitamin B<sub>1</sub> deficiency and the B.B.S. in the blood. Polyneuritis of pigeons developed before the elevation of the B.B.S., and in one group of animals the disappearance of symptoms preceded the lowering of the B.B.S. Furthermore, symptoms of chronic polyneuritis were not accompanied by a rise in the B.B.S. in the blood.

Vitamin B<sub>1</sub> deficiency in certain of the patients studied was associated with disturbance both of the fat and of the carbohydrate metabolism. This was shown not only by the increase in carbonyl substances other than acetone and diacetic acid, but also by hyperglycemia, which disappeared following treatment.

#### SUMMARY AND CONCLUSIONS

1. Measurement of the B.B.S. in the blood gives a quantitative index of metabolic disturbances which result in an accumulation of carbonyl

compounds. It is not possible, however, to differentiate the various carbonyl metabolites by this procedure.

2. Observations are reported on the relation of B.B.S. to other chemical constituents of the blood, such as glucose, nonprotein nitrogen and carbon dioxide capacity.

3. Since an increase in the B.B.S. occurs in a variety of diseases, its use as a diagnostic test of any one disease is not feasible.

4. The B.B.S. in the blood is increased in vitamin B<sub>1</sub> deficiencies, in unregulated diabetes mellitus, in febrile infections, in severe congestive circulatory failure due to organic heart disease, and in certain less common diseases.

5. In diabetes mellitus, incomplete fat metabolites, such as acetone and diacetic acid, contribute a portion to the increase in the B.B.S. Pyruvic acid is present in the urine of some patients with unregulated diabetes. The level of the B.B.S. is an accurate index of the degree of ketosis and of the clinical severity in this disease.

6. The elevation of the B.B.S. in certain cases of vitamin B<sub>1</sub> deficiency cannot be entirely explained by the presence of acetone, of diacetic acid or of pyruvic acid. The effect of vitamin B<sub>1</sub> in lowering the B.B.S. in such cases suggests an important oxidative rôle of this vitamin in metabolism.

7. In alcoholic polyneuritis and heart failure ("alcoholic beriberi") alcohol *per se* is not responsible for elevation of the B.B.S.

8. In 4 patients with vitamin B<sub>1</sub> deficiency hyperglycemia was observed which disappeared under treatment.

This investigation was carried out with the technical assistance of Miss Jane W. Bryant, A.B., and Miss Sophia M. Simmons, S.B.

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# THE ESTIMATION OF THE SUBCUTANEOUS TISSUE PRESSURE BY A DIRECT METHOD<sup>1</sup>

By GEORGE E. BURCH AND WILLIAM A. SODEMAN

(From the Department of Medicine, Tulane University of Louisiana, and the Charity Hospital of Louisiana, New Orleans)

(Received for publication May 12, 1937)

Subcutaneous tissue pressure may be defined as the pressure with which the tissue structures resist any change in their anatomical relationships. Since the pioneer work of Landerer (1) in the estimation of tissue pressure, little has been done to evaluate the importance of this factor in normal and pathological physiologic processes. Recent studies (2, 3, 4) have shown no uniformity in normal values. The present investigation is an attempt to throw additional light upon these problems and establish standards for our technic.

## METHODS AND MATERIALS

We employed a simple, direct, manometric method for the determination of tissue pressure, which is based upon the modification by Henderson, et al. (5) of Rimi's technic for the determination of muscle tone. The apparatus (Figure 1) consists essentially of a U-tube water ma-

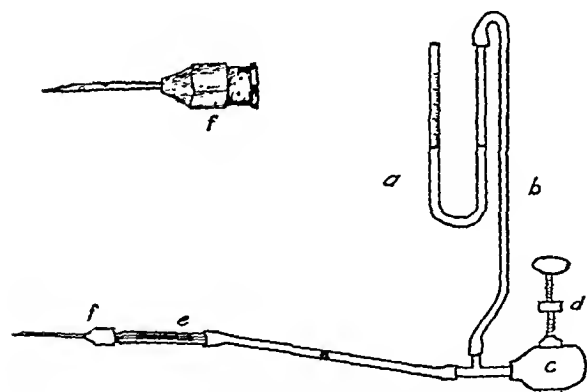


FIG. 1. APPARATUS USED IN THE DETERMINATION OF TISSUE PRESSURE (SEE TEXT)

nometer (a) which is connected by rubber tubing (b) to a rubber pressure bulb (c) controlled by a screw clamp (d) and also connected to a 1 mm. bore glass adapter (e) to which is fastened a 26-gauge needle (f). Parallel lines were etched at millimeter intervals on the wall of the adapter so as to facilitate reading slight movements of a meniscus. The opening in the beveled end of the needle was occluded with solder and four open-

ings symmetrically drilled into the lumen through the wall of its distal third. In use, sterile normal saline was drawn into the needle and about half way up the adapter, and the pressure in the system was then brought to atmospheric. The needle was then inserted into the subcutaneous tissues of the part to be studied. The pressure within the system was slowly raised by the screw clamp until the meniscus in the adapter just began to move. This pressure was taken as the subcutaneous tissue pressure. Three readings were taken which agreed within  $\pm 1$  mm. for each determination. All determinations were made under aseptic conditions. At no time did we inject more than 0.5 cu. mm. of saline and usually not more than 0.1 cu. mm.

## RESULTS

We are reporting results on normal subjects and patients with cardiac edema. A preliminary report of the results on normal subjects has already been published (6).

In 10 normal individuals the subcutaneous tissue pressure was determined at heart level in four common sites of edema formation, dorsum of the hand, volar surface of the forearm, pretibial area and dorsum of the foot. The subjects rested in the supine position for 15 minutes and the determinations were made in the order given. The results are summarized in Table I. The mean val-

TABLE I  
Subcutaneous tissue pressure at heart level in normal subjects

Subject number	Age	Sex	Dorsum of hand	Volar surface of forearm	Pretibial area	Dorsum of foot
	years		mm. H <sub>2</sub> O	mm. H <sub>2</sub> O	mm. H <sub>2</sub> O	mm. H <sub>2</sub> O
1	23	M.	28	11	22	35
2	49	M.	12	29	22	36
3	24	M.	19	26	23	24
4	25	F.	17	23	54	15
5	25	M.	8	40	54	43
6	29	M.	15	19	45	36
7	27	F.	22	18	40	38
8	23	M.	14	17	18	18
9	26	M.	14	14	43	25
10	48	M.	30	39	50	38
Mean			17.9	23.6	37.1	30.8
Maximum			30	40	54	43
Minimum			8	11	18	15

<sup>1</sup> Presented before the American Society for Clinical Investigation at Atlantic City on May 3, 1937.



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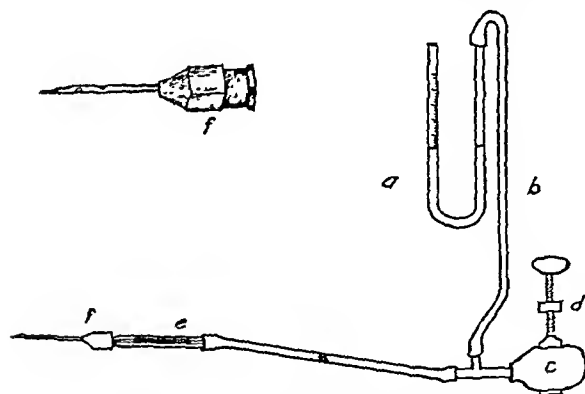


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3	24	M.	19	26	23	24
4	25	F.	17	23	54	15
5	25	M.	8	40	54	43
6	29	M.	15	19	45	36
7	27	F.	22	18	40	38
8	23	M.	14	17	18	18
9	26	M.	14	14	43	25
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Mean			17.9	23.6	37.1	30.8
Maximum			30	40	54	43
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ues at heart level varied from 17.9 to 37.1 mm. of water, the values being slightly higher in the lower extremities. The individual determinations varied from 8 to 54 mm. of water.

After completing the above measurements, the patient stood up and a determination was made in the dorsum of one foot while all the body weight was borne on the other foot. The pressure in the dorsum of the foot was increased in assuming the erect position in seven of the eight subjects studied, the greatest increase in any subject being 30 mm. of water. Two of the subjects refused a fifth puncture.

When the body weight was borne equally upon both feet and the measurement of tissue pressure was repeated in the previously relaxed foot without withdrawal of the needle, the values invariably decreased. If the needle was withdrawn between these two observations, the values were increased (Table II). The latter determinations are the more accurate. Such technical difficulties are considered below.

TABLE II

*Subcutaneous tissue pressure of the dorsum of the foot in normal subjects. Immediate effect of standing and bearing weight*

Subject number	Age	Sex	Standing without bearing weight	Standing and bearing body weight	
				Needle not removed	Needle removed and reinserted
	years		mm. H <sub>2</sub> O	mm. H <sub>2</sub> O	mm. H <sub>2</sub> O
11	33	M.	76	56	100
12	23	M.	31	30	38
13	26	M.	66	50	82
14	26	M.	42	12	102
Mean			53.7	37.0	80.5

The immediate effects of elevated venous pressure were studied in the dorsum of the hand in the 10 subjects of Table I. With the hand at heart level, the venous pressure was increased by inflating a blood pressure cuff around the arm in steps of 135 mm. of water (10 mm. Hg) at two minute intervals. In no instance was the diastolic blood pressure exceeded. The results are illustrated in Table III. It can be seen that increases in venous pressure over short intervals of time had relatively slight effect on the tissue pressure readings.

TABLE III

*The immediate effects of elevation in venous pressure on the determination of tissue pressure in the dorsum of the hand in normal subjects at heart level*

Subject number	Pressure in cuff constricting arm (mm. H <sub>2</sub> O)					
	0	270	405	540	675	810
1	28	14	20	22	18	20
2	12	26	23	42	45	60
3	19	16	16	18	26	28
4	17	12	12	9	14	17
5	8	8	8	10	11	12
6	15	20	20	22	33	20
7	22	19	18	14	14	*
8	14	14	14	18	19	16
9	14	16	20	16	18	20
10	30	40	42	42	46	45
Mean	17.9	18.5	19.3	21.3	24.4	26.4

\* Patient's diastolic blood pressure was 60 mm. Hg.

The effects of increased venous pressure over longer periods of time were investigated in six normal subjects standing quietly against a table inclined at 75° for one hour. Measurements were made at the beginning and end of the hour. The results are presented in Table IV. The mean value for the six subjects was 53.5 mm. of water at the beginning and 65.2 at the end of the hour. The greatest increase in any individual was 31 mm. of water. In one instance (Subject 19) the final reading was taken after 37 minutes, just before the patient fainted. Pitting edema did not develop in any subject.

Determinations were made upon ten patients with congestive heart failure and increased venous pressure. Patients were chosen only when edema was increasing, as determined by history and inspection, and these patients were followed when possible until they became edema free. All de-

TABLE IV

*The effect of standing one hour on the subcutaneous tissue pressure in the dorsum of the foot of normal subjects*

Subject number	Age	Sex	Pressure at beginning of hour	Pressure at end of hour
			mm. H <sub>2</sub> O	mm. H <sub>2</sub> O
15	23	M.	75	82
16	23	M.	58	60
17	48	M.	94	104
18	46	M.	44	52
19	31	M.	28	59*
20	20	M.	22	34
Mean			53.5	65.2

\* Patient fainted after standing 37 minutes. Reading was made just before he fainted.

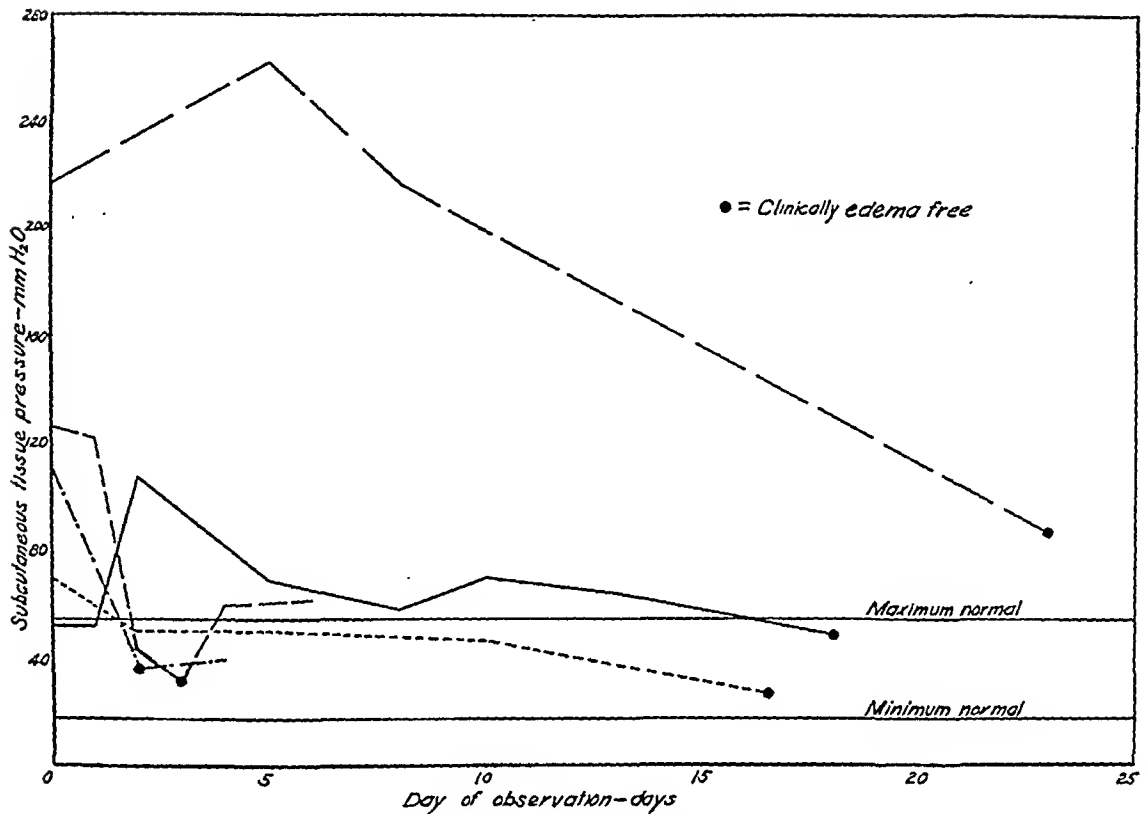


FIG. 2. SUBCUTANEOUS TISSUE PRESSURE (PRETIBIAL AREA) IN 5 PATIENTS WITH CARDIAC FAILURE

terminations were made in the pretibial area in the supine position in patients confined to bed. First determinations varied from 47 to 218 mm. of water, 9 of the 10 measurements being above 54 mm. of water which was the highest value observed in our series of normals. Whether the value was high or low could be roughly estimated in these individuals by the appearance and firmness of the part to inspection and palpation. Figure 2 illustrates the course of the tissue pressure readings in five of the subjects followed. A great variation in the course of the pressures from patient to patient is evident. The highest value obtained in the entire group was 262 mm. of water. In the ten patients the highest values were 188, 126, 60, 242, 116, 156, 110, 262, 70, and 58 mm. of water, respectively. In general, the readings followed the clinical course of the edema. The factors influencing these changes are analyzed in the discussion.

#### DISCUSSION

The importance of tissue pressure in the mechanism of fluid exchange in the body has just

recently been recognized (2, 7, 8). Indirect estimations of the tissue pressure have appeared in the American literature, but we have been unable to find records of direct determinations reported in this country. The results vary widely. Landerer (1), by a direct manometric method found that the subcutaneous tissue pressure was 550 mm. of water in the thigh of man in the sitting position. Gildemeister and Hoffmann (3) by an indirect method with the use of an elastometer, calculated the subcutaneous tissue pressure to be 130 mm. of water. Such indirect methods, as Landis and Gibbon (7) state, provide no information concerning the absolute tissue pressure. Meyer and Holland (4), with a direct manometric method, found a normal intracutaneous tissue pressure of 55 to 85 mm. of water and a subcutaneous tissue pressure of 20 to 40 mm. of water. Youmans and his associates (2), with an indirect method, calculated that the tissue pressure of the lower extremity when standing varied from 311 to 487 mm. of water. Smirk (9), in his studies on the causes of edema in congestive heart failure, found

by a manometric method edema fluid pressures varying from 40 to 180 mm. of water.

The great variations in the reported tissue pressure values are probably due to differences in technic. Any method which directly measures the tissue pressure may introduce errors. Our method was chosen because, as far as we could determine, it minimized error more than any technic previously described. The introduction of the needle and saline into the subcutaneous tissues tends to raise the tissue pressure. The fact that we use a small needle and inject a negligible amount of saline minimizes this effect. The tearing of tissue by the needle would tend to lower the tissue pressure. This effect is again minimized by the size of the needle employed. These two factors tend to nullify each other. Once the needle is inserted it must be maintained in position and the relationship of tissues to each other must not be changed. With movement of the part without withdrawal of the needle the change in pressure is due to at least two factors, a change in the natural relationship of tissues to each other and an interference with this change by the presence of a rigid immobile structure. Following movement of the part, withdrawal and reinsertion of the needle will eliminate this latter effect. The magnitude of such errors is illustrated in Table II. With care and experience such errors may be avoided. In five subjects repeated determinations were made in the same area with withdrawal of the needle. In three instances the determinations were made on separate days. The maximum variation for each subject was  $\pm 4$  mm. of water, except in one instance in which the part could not be kept immobile and the variations were  $\pm 7$ .

Our values for subcutaneous tissue pressure at heart level for normal individuals (Table I) agree in the main with those of Meyer and Holland (4). This pressure and the colloid osmotic pressure of the plasma are two important known antilitering pressures which tend to neutralize the filtering pressure (hydrostatic pressure and colloid osmotic pressure of the tissue fluids). Since other factors may be important in the movement of fluid through the capillary wall, we do not feel justified in formulating an equation to quantitate these values as has been done by others (2, 4). This is particularly true in the light of Smirk's (9) obser-

vations that capillary permeability is a variable and significant factor. Smirk's observations clearly indicate that the formula of Youmans et al. (2) is based upon erroneous assumptions. Smirk has shown that with increased venous pressure, filtering and antilitering pressures have not reached an equilibrium even at five hours. Youmans' calculations are based upon the assumption that an equilibrium is reached at the end of one hour. Other factors such as the filtration rate, variations of the capillary bed within the part, colloid osmotic pressure of the tissue fluids, and lymph flow, which are pointed out by Smirk as important factors, are not considered in Youmans' formula. It is evident that the number of unknown factors which enter into the interchange of fluid between blood vessels and tissues is so great that a workable formula is impossible.

It is well known that an elevation in venous pressure disturbs the equilibrium between filtration and antiliteration through the capillary membrane, favoring an accumulation of interstitial fluid. The effect of this disturbance upon the tissue pressure was studied in three ways, (1) by elevation of the venous pressure for short periods of time with a blood pressure cuff, (2) by the influence of standing for one hour on determinations in the foot, and (3) by the effect of the prolonged increased venous pressure of congestive heart failure. It is evident from the data that an elevation of venous pressure for a short period of time produced no marked change in the determinations of tissue pressure. This observation is in keeping with those of Smirk, who found that an equilibrium was not established up to five hours. Since equilibrium is not established in one hour, any calculation of tissue pressure from a formula would give results that are too high (2). This explains the discrepancy between calculated values and our determinations. Prolonged elevation of venous pressure (congestive heart failure) was accompanied by a definite increase in tissue pressure. This will be discussed later.

The factors which vary tissue pressure when venous pressure is elevated are not clearly understood. There are at least three important variables, (1) the change in filtration rate, (2) distensibility of the surrounding tissues, and (3) the rate with which interstitial fluid is removed, for example, through lymphatics. Since only meas-

urements of the first variable have been made, valid calculations of tissue pressure up to this time have been impossible, so that an accurate estimation of tissue pressure is possible only by direct measurement.

We found that the height of the arterial pressure had no direct influence upon the magnitude of the tissue pressure. It is interesting to point out that determinations of intravascular pressure by collapse technic are in error by the value of the tissue pressure. This error is small for high (arterial) pressures, but may become large in low (capillary, venous) pressures. Since the tissue pressure is unaffected by hypertension, comparison of capillary pressure by collapse technic in normal and hypertensive individuals is valid (10).

In increasing cardiac edema, we invariably found elevated subcutaneous tissue pressure. These findings are in accord with those of Smirk (9). These values depend upon the three variables previously mentioned, (1) increase in filtration rate, (2) distensibility of the surrounding tissues, and (3) the rate at which interstitial fluid is removed. In cardiac edema, interstitial fluid is accumulating more rapidly than it is being removed. As this fluid accumulates, the tissues are displaced and the fibrous network is stretched, the tissue tension increases and this in turn alters the filtration and removal rates. The interplay of these three factors influences the level to which tissue pressure may rise. It is possible that prolonged elevated tissue tension may overstretch these fibers so that with reduction of venous pressure and removal of edema they will not immediately return to their original state (7). Such a situation could explain the low tissue pressure found in receding edema and the post-edematous period (Figure 2). Such changes could explain the variable results of Meyer and Holland. In one instance the administration of salyrgan produced profuse diuresis resulting in a marked decrease of the edema and the tissue pressure as well. Diuretics, therefore, may influence tissue pressure.

In three of the patients with cardiac edema, the venous and tissue pressures were observed simultaneously. It was noted that the return of the tissue pressure to normal lagged behind the venous pressure. At no time did the tissue pressure reading exceed the venous pressure determination.

Should tissue pressure exceed venous or capillary pressure, collapse of these vessels would ensue.

Values for non-cardiac edema will be reported later.

#### SUMMARY AND CONCLUSIONS

Using a direct manometric method for the determination of tissue pressure, we found in 10 normal individuals mean values at heart level varying from 17.9 to 37.1 mm. of water in the subcutaneous tissue of the dorsum of the hand and foot, volar surface of the forearm and the pretibial area. The tissue pressure in the dorsum of the foot was increased in the erect position and further increased by weight bearing. Normal tissue pressure was less than the accepted values for capillary and venular pressure.

The full effect of increased venous pressure on tissue pressure was not immediate; up to one hour the effect was slight. In congestive heart failure with prolonged high venous pressure the tissue pressure was greatly elevated.

In 10 patients with increasing cardiac edema, the tissue pressure was definitely increased. In receding cardiac edema the values were lowered, at times even below normal, to return finally to a normal level.

Tissue pressure is an important factor in the control of movement of fluid between blood vessels and tissue spaces. Factors other than venous pressure are important in the regulation of tissue pressure.

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# CLINICAL STUDIES OF THE BLOOD VOLUME. III. CHANGES IN BLOOD VOLUME, VENOUS PRESSURE AND BLOOD VELOCITY RATE IN CHRONIC CONGESTIVE HEART FAILURE

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Knowledge of the changes in blood volume taking place in the course of congestive heart failure is necessary for an understanding of alterations in the dynamics of circulation in that disease. Changes in blood volume in cardiac decompensation reported in the literature are of a conflicting nature. Bock (1), Brown and Rowntree (2), Thompson (3), Schürmeyer (4), Mies (5), using modifications of the original dye method of determining the blood volume; Plesch (6), using the carbon monoxide method, and Ewig and Hinsberg (7); using a combination of the two methods, have reported the blood volume as variably increased during cardiac decompensation. The most extensive studies have been made by Wollheim (8, 9, 10, 11), using a modification (12) of the original Keith-Rowntree technique, Levin (13), and Goldbloom and Libin (14). On the basis of their studies these authors have described two types of congestive failure; the so-called "plus" and "minus" types. In the former type, in which failure is characterized by cyanosis, distension of cervical veins, engorgement of the liver, edema and other symptoms and signs of congestion, the volume is said to be above normal during failure and to decrease with compensation. In the latter type, characterized by pallor, poor venous filling, marked respiratory distress and weakness, but no marked edema, the volume is said to be below normal during failure and to increase during compensation.

In this communication we will report the results of our studies in patients in congestive heart failure, selected from the Cardiac Clinic and Medical Wards of the Peter Bent Brigham Hospital. We have employed the method developed by Gregersen, Gibson and Stead (15), as modified for clinical use by us (16). This method measures the plasma volume by means of an azo dye, Evans Blue, and employs the spectrophotometer for colorimetric estimation of dye concentration in

serial samples of blood serum. The total blood volume and red cell volume are calculated from hematocrit values of venous blood and the plasma volume. In addition, many observations on venous pressure and circulation time were made by methods previously described (16).

Changes occurring during the transition from the compensated to decompensated state were studied by means of statistical analysis of a large number of single determinations in patients in varying stages of heart disease. Cases were placed in groups according to the severity of symptoms and clinical signs of congestive failure. Changes in the average values of these groups were taken as characteristic of the trend of blood volume, hematocrit, venous pressure and circulation time in progressive congestive failure.

Changes taking place during recovery from decompensation, or during further progression of heart failure were studied by means of repeated determinations in the same individual during the course of his treatment for congestive heart failure in the hospital, or after discharge therefrom. Both methods have certain limitations, but yield results which are mutually complementary and confirmatory and from which valid conclusions as to the true state of circulatory dynamics in heart disease may be drawn.

One of the principal difficulties in a study of this sort is the selection of normal values for comparative purposes. Normal values for venous pressures, circulation times and hematocrits fall within clearly definable limits, and for this study we have employed the average normal values found by us by the methods employed as previously reported (17). The normal blood volume for a given individual can, however, be assigned with less certainty. In a previous communication (17), we stated that the blood volume varied within wide limits in normal persons of varying habitus and proportion of muscle and

fat. We also stated that, within certain limits, total blood volume in normal persons bore a relationship to height, weight and surface area. It was our opinion that the average of normal values based on either height or surface area obtained in fairly large groups of individuals might offer a reasonable basis for showing changes from the normal volume levels occurring in disease.

It is obvious that weight is altered in certain phases of cardiac disease, owing to the presence of edema fluids during the congestive phase, and also to the cachexia accompanying chronic heart disease, to such a point as to be meaningless for normal volume estimation on the basis of surface area or weight. We have therefore used the least variable of physical measurements, namely, height, as the basis for estimation of normal blood volume in these studies. This procedure has the advantage over using the average total blood volume of a large number of "normal" individuals of varying age, type of build and state of nutrition, of more closely estimating normal volume with respect to the size of the individuals comprising the different groups. While it must be clear that such normal values for individuals only approximate their true basal volume before decompensation set in, we feel that comparison of average deviations from normal of similar groups shows trends of real significance. Similarly, in individuals, increases or decreases of a range greatly in excess of predicted normal values may be taken as evidence of significant changes.

#### *Changes accompanying progressive degrees of congestive heart failure*

Single determinations were made in 99 individuals with clinically proven organic heart disease. Blood volume, venous pressure and circulation time were determined in the basal state in each case. Cases were divided into four groups.

Group I contained patients with valvular heart disease who exhibited no symptoms or signs of cardiac insufficiency. There were seven males and eleven females in this group; with the exception of three females with congenital heart disease all had valvular lesions diagnosed as being of rheumatic origin.

In Group II were placed those patients who had symptoms of cardiac insufficiency; dyspnea

on moderate effort, easy fatigue with or without actual limitation of activity, but in whom no physical signs of congestive failure were observed. There were 12 males and 6 females in this group, all of whom had rheumatic valvular disease except one male with luetic aortic insufficiency.

Groups III and IV consisted of patients in frank congestive failure; those with venous pressures above 150 mm. of water being placed in the latter group, as exhibiting the most severe degree of decompensation. Group III contained 12 males and 10 females; 5 of each sex had rheumatic heart disease, one male syphilitic aortic insufficiency, 2 males and one female hypertensive heart disease and the remainder chronic myocarditis. In Group IV were 7 males and 8 females; 2 males and 6 females had rheumatic

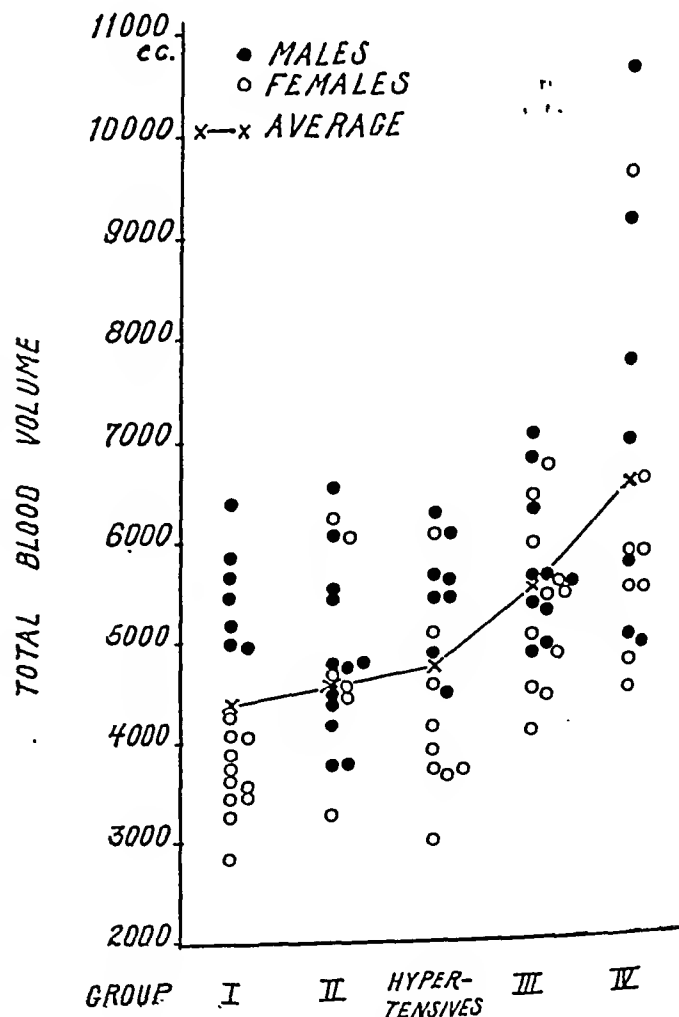


FIG. 1. INCREASE IN ABSOLUTE TOTAL BLOOD VOLUME IN PROGRESSIVE HEART FAILURE

While a considerable spread in individual volumes occurs the trend of average values indicates a considerable increase in volume over normal levels in congestive heart failure.

heart disease, 2 males hypertensive heart disease, 1 female constrictive pericarditis of unknown etiology and the remainder chronic myocarditis.

A separate grouping was made of sixteen patients with essential hypertension, of whom 9 were males and 7 females, in whom there was no evidence of renal or cardiac involvement. A few of these complained of fatigue and voluntary restriction of activity, but because of the difficulty of ascribing these symptoms to cardiac disability no attempt was made to class these patients in either of the first two groups. During the period of observation the systolic blood pressure was above 150 mm. of mercury.

TABLE I

*Changes in blood volume, hematocrit, venous pressure and circulation time in progressive stages of congestive heart failure*

Group	Number of cases		Average predicted total blood volume based on height	Total determined blood volume		Average values for groups			
	Males	Females				Deviation from predicted normal	Hematocrit	Venous pressure	Circulation time
I	7	11	cc. 4567	cc. 4348	cc. 79.0	per cent - 4.8	per cent of cells 41.1	mm. H <sub>2</sub> O 77	seconds 17.5
II	12	6	4336	4524	76.7	+ 4.4	45.2	75.5	24.2
Hypertensives	7	9	4573	4714	67.9	+ 2.7	43.9	77.5	20.0
III	12	10	4656	5600	91.5	+22.3	44.4	107	37
IV	7	8	4264	6543	97.0	+55.3	45.6	206	46.7

The results of these observations are presented in Figure 1 and Table I. While in each group there was a considerable spread in the individual values for blood volume, hematocrit, venous pressure and circulation time, the trend of the average values for each of these groups, namely, a progressive increase above normal with advancing decompensation is definite.

The average percentage deviation from normal of each group is shown in Figure 2. The value for Groups I and II may be considered to be within normal limits. With the onset of physical signs of congestive failure (Group III) a large increase above normal, amounting to 22.3 per cent occurs, while in severe failure (Group IV) the increase above normal is 55.3 per cent. In

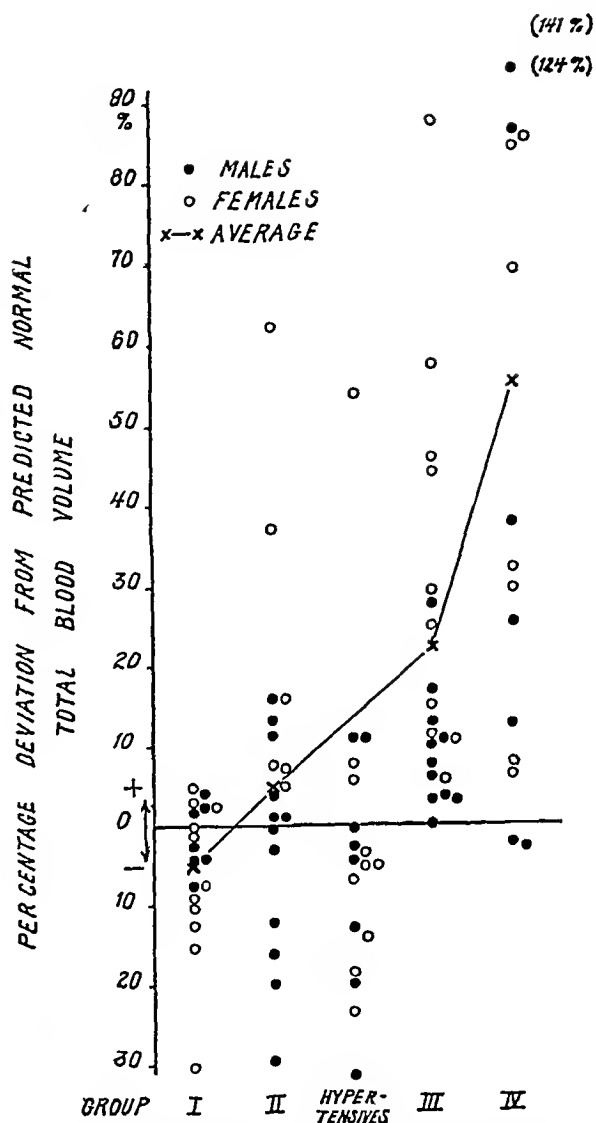


FIG. 2. THE PERCENTAGE INCREASE OF AVERAGE FIGURES ABOVE PREDICTED NORMAL BLOOD VOLUME IN PROGRESSIVE CONGESTIVE HEART FAILURE

A definite increase occurs in Group II, preceding the development of physical signs of congestion. The average percentage above predicted normal total blood volume for the group of compensated hypertensives is 2.7 per cent, and for five hypertensives in congestive failure (included in Groups III and IV), is 36.2 per cent.

the hypertensive group the average value for patients exhibiting no signs of failure, is well within normal limits. In hypertensive patients in failure, however, the total volume is above normal limits, the average elevation above normal of the 5 patients studied being 36.2 per cent.

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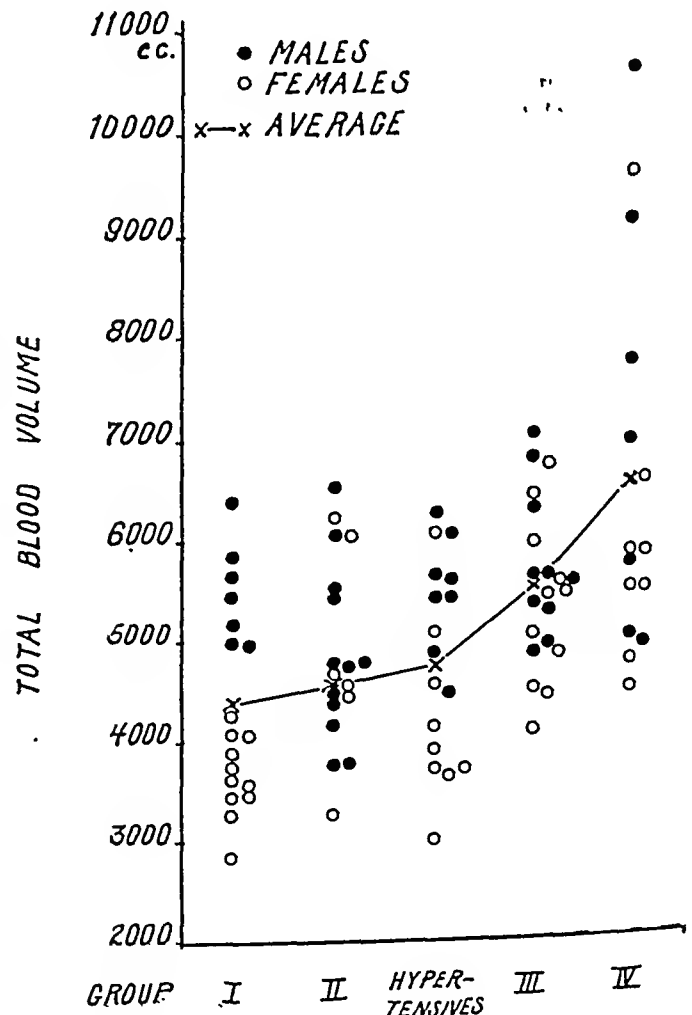


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			cc.	cc.	cc. per kgm.	per cent	per cent of cells	mm. H <sub>2</sub> O	seconds
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II	12	6	4336	4524	76.7	+ 4.4	45.2	75.5	24.2
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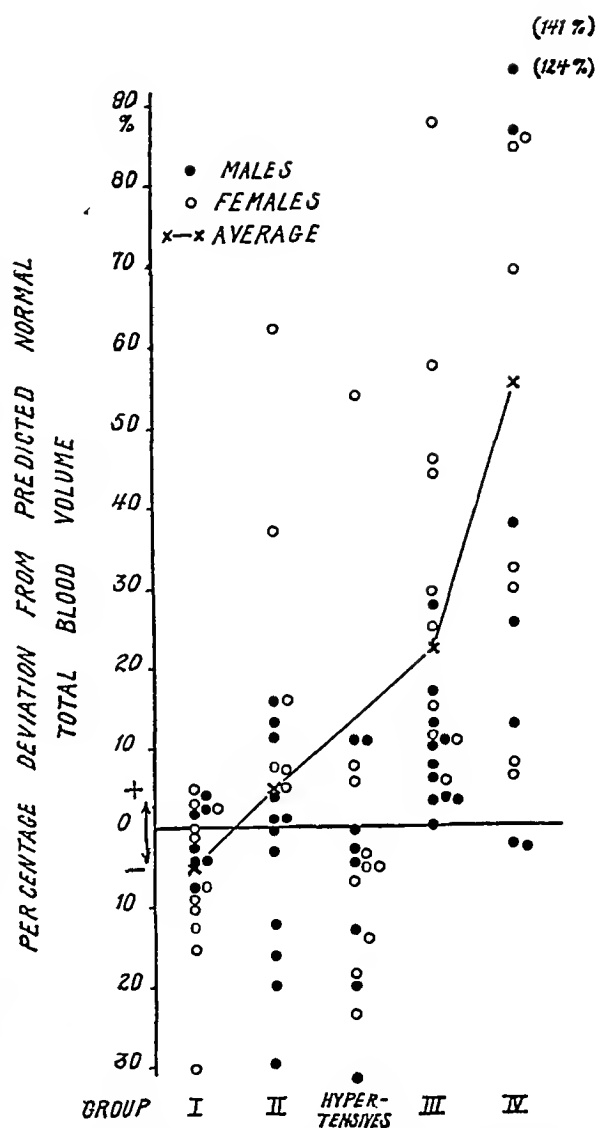


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TABLE II

*Relation of total blood volume and hematocrit to venous pressure and circulation time*

Venous pressure	Number of cases		Average predicted total blood volume	Total blood volume	Deviation from predicted normal total volume	Hematocrit
	Males	Females				
mm. H <sub>2</sub> O			cc.	cc.	per cent	per cent of cells
0-74	11	12	4542	4800	+ 1.29	43.7
75-99	10	12	4662	4700	+ 0.9	44.6
100-149	17	7	4874	5730	+17.6	44.6
150+	7	8	4264	6543	+53.5	44.4

Circulation time	Number of cases		Average predicted total blood volume	Total blood volume	Deviation from predicted normal total volume	Hematocrit
	Males	Females				
seconds			cc.	cc.	per cent	per cent of cells
0-19	7	16	4434	4330	+ 3.6	41.7
20-29	17	1	4790	4960	+ 3.7	44.8
30-39	5	9	4335	5564	+28.3	43.6
40+	5	7	4460	5921	+32.7	44.9

The relationship of changes in absolute volume to venous pressure and circulation time is shown in Table II, from which it is apparent that, in general, marked increases in volume take place only when the venous pressure is in excess of 100 mm. and the circulation time slower than 20 seconds; and that the degree of elevation in blood volume is directly related to the degree of rise in venous pressure and slowing of the circulation time. It is also apparent that in the increased volume of failure the rise is not equally shared by the plasma and red cell portions, the increase in hematocrit values from Groups I to IV suggesting a slight concentration of the blood as failure progresses.

*Changes accompanying restoration of compensation or progression of decompensation*

For this portion of our study serial observations were made in patients in varying degrees of diac decompensation while under treatment in hospital wards. Clinical grouping of these has been made, according to the course of

the patient's disease; those showing marked improvement; those undergoing fluctuating courses, characterized by periods of improvement and relapse; and those in whom the disease progressed to a fatal termination.

In Table III is shown the course of blood volume, venous pressure, circulation time, and hematocrit in 13 patients in congestive failure in whom bed rest, digitalization and diuresis were accompanied by a return to a compensated state. The underlying etiology in the failure of these patients is as described in the table. In every case the blood volume at the time of the first determination, made at the time of admission to the hospital, was in excess of the normal volume based on body height for that individual, the extremes in elevation ranging from 5.7 to 85.7 per cent. The average value for the entire group is 29.6 per cent above normal.

In all these 13 cases, of varied etiology, a striking decrease in blood volume, venous pressure and a decrease in circulation time occurred during compensation. Decreases in total volume amounted to a liter or more in the majority of cases, the extremes ranging from 220 cc. (Number 56, Table III) to 3950 cc. (Number 110, Table IV). In general, those patients experiencing the greatest clinical improvement as evidenced by clinical signs, alleviation of symptoms, weight loss and diuresis, had the largest reductions in total volume from failure levels.

The reduction in plasma volume was at first proportionately greater than the reduction in red cell volume. In 9 of the 13 cases summarized in Table III, hematocrit values rose as compensation was restored. Thus, as illustrated in Cases 55, 62, and 147 (Table III), with clinical improvement there was a considerable fall in plasma volume accompanied by a moderate decrease in red cell volume with resultant rise in hematocrit, the change taking place in a comparatively short time. With further improvement, the plasma volume fell but little more, while the red cell volume continued to diminish with resultant decline in hematocrit value.

The reduction in red cell volume varied greatly, a decrease of about 500 cc. taking place during the transition from failure to compensation. The largest reduction encountered was in Case 110 (Table IV), amounting to 1410 cc. in a period of

TABLE III

*Observations on 13 patients experiencing distinct clinical improvement under treatment for congestive heart failure*

Case number	Age	Date	Diagnosis	Height cm.	Weight kgm.	Predicted total blood volume based on height cc.	Venous pressure mm. H <sub>2</sub> O	Circulation time sec-onds	Hemato-crit per cent of cells	Blood volume				Deviation from predicted normal total volume per cent
										Plasma cc.	Red cell cc.	Total cc.	per kgm.	
36 Male	56	June 10, 1935 July 10, 1935	Chronic myocarditis, generalized arteriosclerosis, hypertension	163.8	55.6 50.2	4890	65 100	64 43	43.7 47.9	3160 2700	2460 2480	5620 5180	101.3 101.5	+14.9 + 5.9
38 Male	72	June 14, 1935 July 5, 1935	Chronic myocarditis, arteriosclerosis, auricular fibrillation	170.2	66.6 58.2	5320	100 40	37 33	45.9 45.0	3690 3200	3110 2620	6800 5820	102.7 100.0	+27.8 + 9.4
50 Female	25	June 27, 1935 July 10, 1935 Jan. 10, 1936	Rheumatic heart disease, mitral stenosis and insufficiency, aortic insufficiency, auricular fibrillation	157.5	52.2 43.9 53.5	3850	185 110 85	57 60 25	50.9 52.5 44.5	2800 2700 2560	2880 2970 2050	5680 5670 4610	108.8 120.0 83.0	+47.5 +47.3 +19.8
55 Male	51	July 2, 1935 July 9, 1935 July 24, 1935	Chronic myocarditis, auricular fibrillation, angina pectoris, arteriosclerosis	169.0	94.6 80.2 81.0	5250	265 55 40	60 38 28	49.8 57.6 51.9	3890 2800 3100	3860 3810 3210	7750 6610 6310	81.9 82.2 77.9	+47.6 +25.9 +22.1
62 Male	46	July 9, 1935 July 16, 1935 July 25, 1935	Chronic myocarditis, hypertension, auricular fibrillation	162.5	61.6 56.2	4780	140 60	30 24	40.5 39.7	3150 3060	2140 2010	5290 5070	86.2 90.2	+10.7 + 6.1
77 Female	63	Oct. 12, 1935 Nov. 7, 1935	Rheumatic heart disease, mitral stenosis and insufficiency	167.6	71.4 66.4 65.0	5160	200 70 50	67 44 39	54.7 56.1 51.2	3160 2530 2480	3820 3240 2350	6980 5770 4830	97.8 86.9 74.3	+35.3 +11.8 - 6.4
87 Male	63	Nov. 3, 1935 Nov. 10, 1935	Rheumatic heart disease, mitral stenosis and insufficiency, hypertension	157.5	61.0 57.8	3850	90 90	28	46.1 50.3	2190 1650	1880 1670	4070 3320	66.7 57.3	+ 5.7 -13.1
147 Male	48	Jan. 22, 1936 Feb. 4, 1936 Feb. 18, 1936	Rheumatic heart disease, mitral stenosis and insufficiency, auricular fibrillation, hypertension	172.0	72.2 72.0	5320	100 70	60 38	41.9 44.3	3660 3160	2650 2530	6310 5690	87.4 79.1	+18.6 + 7.0
162 Male	45	March 27, 1935 April 23, 1936	Chronic myocarditis, arteriosclerosis	161.3	59.2 51.8 52.0	4660	165 85 30	37 43 27	49.3 51.9 51.3	2540 2200 2200	2460 2370 2320	5000 4570 4520	84.4 88.1 86.8	+ 7.3 - 0.9 - 3.0
169 Female	29	April 11, 1936 April 21, 1936	Rheumatic heart disease, aortic insufficiency, hypertension	185.4	101.4 87.2	5820	145 75	18 29	31.3 29.8	5060 4350	2310 1850	7370 6200	72.6 71.1	+26.8 + 3.5
188 Female*	50	June 2, 1936 Dec. 16, 1936 Jan. 15, 1937 May 27, 1937	Rheumatic heart disease, mitral stenosis and insufficiency	163.8	63.8 56.4	4010	160 80	45 40	48.0 46.2	2420 2310	2310 1990	4730 4300	72.7 76.2	+48.0 +46.2
189 Female	19	Jan. 2, 1936 Jan. 19, 1936	Constrictive pericarditis	147.3	66.2 63.0 58.2 60.0	3150	290 122 104 90	50 24 24 28	44.1 44.5 41.0 43.0	3270 3010 2920 2595	2580 2020 2030 1963	5850 5430 4950 4560	88.3 86.2 85.2 75.9	+85.7 +72.5 +50.7 +44.7
				160.0	56.6 49.4	3930	195 55	44 27	42.3 43.4	3140 2540	2350 1960	5490 4500	97.1 91.0	+39.7 +24.7

\* The second to fourth studies in the patient were made following successful surgical freeing of the pericardial adhesions.

14 days. In none of the cases studied was compensation accompanied by an increase in either plasma or total blood volume.

In contrast, Table IV presents serial studies in 3 cases in whom recovery was not continuous but was interrupted by a relapse to a more severe degree of failure. During these periods of in-

creased failure of the circulation, as indicated by clinical signs and symptoms, and frequently by increases in venous pressure and in circulation time, there was an increase in plasma and total volume over levels previously attained during periods of improvement.

Serial studies in 5 cases in whom such fluctuation

tuating courses terminated fatally are presented in Table V. It will be noted that the blood volume remained high or rose as the disease progressed.

## DISCUSSION

Studies of the behavior of Evans Blue after intravenous injection indicate that it does not appear in the pathological transudates of congestive

TABLE IV  
*Observations on 3 patients exhibiting a relapse while under treatment for severe congestive failure*

Case number	Age	Date	Diagnosis	Height	Weight	Pre- dicted total blood volume based on height	Ve- nous pres- sure	Circu- lation time	Hem- ato- crit	Blood volume				Deviation from predicted normal total volume
										Plas- ma	Red cell	Total		
	years			cm.	kgm.	cc.	mm. H <sub>2</sub> O	sec- onds	per cent of cells	cc.	cc.	cc.	cc. per kgm.	per cent
75*	35	Sept. 10, 1935	Rheumatic heart dis- ease, mitral stenosis and insufficiency, auricular fibrillation, hypertension	162.5	86	3980	210	43	41.2	5650	3950	9600	111.3	141.0
		Sept. 24, 1935			75		130	47	48.2	4040	3760	7800	104.0	96.0
		Oct. 9, 1935			76		135	53	50.3	3690	3740	7430	97.5	86.5
		Nov. 28, 1935			70		135	53	49.8	4170	4110	8280	118.2	108.0
110†	23	Nov. 11, 1935	Rheumatic heart dis- ease, mitral stenosis and insufficiency, auricular fibrillation	180.0	71.8	5700	230	54	37.9	6610	4020	10630	148.0	86.4
		Nov. 27, 1935			67.0		85	39	39.1	4240	2610	6850	102.2	20.2
		Dec. 16, 1935			75.6		160	63	37.7	4960	3010	7970	105.2	39.8
		Jan. 8, 1936			61.6		130	38	35.9	4280	2400	6680	108.7	17.2
119‡	50	Dec. 2, 1935	Hypertension, chronic myocarditis, aortic insufficiency	165.0	72.0	4990	105	45	38.1	3700	2270	5970	82.9	19.9
		Dec. 9, 1935			64.8		60	41	42.6	3020	2230	5250	80.8	5.4
		Dec. 16, 1935			65.2		50	56	40.7	3110	2130	5240	80.4	5.2
		Dec. 23, 1935			64.2		140	80	37.0	4160	2460	6620	103.0	32.9
		Jan. 18, 1936			58.4		45	31	36.0	3300	1850	5150	88.3	3.4

\* Some difficulty was experienced in determining the proper maintenance dose of digitalis in this patient. There was a relapse between the 3d and 4th blood volume determinations.

† Suffered a relapse following an acute upper respiratory infection between the 2d and 3d blood volume determinations. As the infection subsided this patient's cardiac status improved.

‡ This patient's course was very fluctuating with periods of improvement and relapse, so that compensation was never well established.

TABLE V  
*Observations on 5 patients with chronic congestive heart failure exhibiting fluctuating clinical courses terminating fatally*

Case number	Age	Date	Diagnosis	Height	Weight	Pre- dicted total blood volume based on height	Ve- nous pres- sure	Circu- lation time	Hem- ato- crit	Blood volume				Deviation from predicted normal total volume	
										Plas- ma	Red cell	Total			
	years			cm.	kgm.	cc.	mm. H <sub>2</sub> O	sec- onds	per cent of cells	cc.	cc.	cc.	cc. per kgm.	per cent	
15 Male*	30	March 8, 1935	Rheumatic heart dis- ease, mitral insuffi- ciency, auricular fibrillation	167.7	52.0	5170	105	63	44.7	2930	2370	5300	101.9	+ 2.5	
		March 3, 1935			52.0		120	38	42.5	3085	2275	5360	103.0	+ 3.7	
		Nov. 3, 1935			52.0		110	83	44.3	2930	2330	5260	101.2	+ 1.7	
		Nov. 20, 1935			48.4		90	42	38.6	2910	1820	4730	97.6	+ 0.9	
16 Male	50	March 11, 1935	Chronic myocarditis, bundle branch block	170.1	59.2	5310		49	35.3	3715	2015	5730	96.8	+ 7.9	
		March 23, 1935			54.8			75	47	31.7	4320	2010	6330	115.2	+19.2
		June 22, 1935			61.2			220	42	38.4	3370	2100	5470	89.5	+ 6.8
		July 17, 1935			58.4			75	35	37.1	3490	2070	5560	95.2	+ 4.7
Sept. 10, 1935	57.2		50	39	37.4	3420	2040	5460	95.5	+ 2.8					
24 Male†	42	May 10, 1935	Syphilitic aortitis, aortic insufficiency, hypertension	168.9		5240		23	43.8	2530	1950	4480	85.1	-14.5	
		May 23, 1935					110	25	38.8	2960	1870	4830	88.2	- 7.8	
49 Fe- male	36	June 26, 1935	Rheumatic heart dis- ease, mitral stenosis and insufficiency	158.8		3890	215	57	50.7	3250	3330	6580	112.2	+67.5	
		July 11, 1935					150	64	50.0	3280	3280	6560	108.4	+67.0	
151 Fe- male	22	Feb. 23, 1936	Rheumatic heart dis- ease, mitral stenosis and insufficiency, aortic insufficiency	157.3		3850	210	30	42.4	2580	1890	4470	104.0	+16.1	
		March 3, 1936					70	34	40.0	2730	1820	4550	109.5	+18.2	

\* This patient underwent the operation of cardiolytic twice during the observation period without relief of symptoms. At no time did he have marked edema.

† This patient had been in chronic failure for 16 years and was extremely cachectic. The predicted normal volume probably represents too high a value.



heart failure. We have been unable to detect the presence of the dye in edema fluid withdrawn from the legs by Southey tube, or in ascitic or thoracic fluid obtained by paracentesis from patients having large amounts of dye in their blood stream at the time the fluids were obtained. Evans and Gibson (18) showed that Evans Blue does not accumulate in the edema fluids of dogs rendered edematous by plasmapheresis even though a high concentration of dye in the blood stream was maintained by repeated injections while the edema was forming. It seems clear therefore that the presence of edema does not invalidate the method of plasma volume determination employed in this study.

We wish to emphasize the fact that many of the patients studied in this investigation were in a condition of chronic congestive failure, many of them having survived one or more attacks of decompensation. Since this disorder is characterized by a progressive wasting of the blood-holding tissues, it is very probable that the "normal" values for many of the individuals, in a state of decline when the first determinations were made, would be considerably lower than those arbitrarily employed in this study. It is therefore probable that, in a given individual, the percentage increase over levels prevailing in a state of compensation to which blood volume rises at the height of congestive failure is far greater than indicated by the percentage increase from estimated normal volume as described above.

That some degree of elevation of blood volume may precede the development of physical signs of congestive failure is suggested by the data given in Figure 1. An average increase of 4.4 per cent above normal occurred in the patients in Group II, none of whom exhibited any physical signs of congestion but did have definite symptoms of limited cardiac reserve.

We are unable to confirm the finding of the existence of two contrasting types of congestive heart failure as regards the state of the blood volume, the so-called "plus" and "minus" types, described by Wollheim (9), Levin (13), and Goldbloom and Libin (14). All of our patients who could be classified as exhibiting the clinical aspects of the so-called "minus" type of failure (Cases 38, 56, 77, and 36), with clinical improvement experienced fair to moderate diureses and

had a decrease in blood volume. Case 77 (Table III) is of particular interest in this regard. This elderly woman with mitral stenosis and hypertension was definitely undernourished, and while the initial volume during frank failure was only slightly above the estimated normal value, the response to therapy was satisfactory, was accompanied by a weight loss of 3.2 kgm. following moderate diureses, and the blood volume underwent a reduction of 750 cc. We conclude that the conception of a "minus" type of failure is a misconception arising from errors inherent in the techniques employed.

The above authors employed various modifications of the original Keith-Rowntree technique using red dyes and the Dubosq or related types of colorimeters. Graff and Clarke (19) showed that hemolysis of samples could give rise to large errors when red dyes were used. H. P. Smith (20) proved that errors amounting to 25 per cent in either direction could be introduced by the presence of residual dye from previous determinations in the "dye-free" sample. Errors in colorimetry when the Dubosq type of colorimeter is used, as shown by Gregersen, Gibson and Stead (15) and Gibson and Evans (16), due to inequality of dye concentration of standard and sample are of such a nature and degree as to give falsely low values in cases with high volumes (in which the dye is greatly diluted so that the standard is more concentrated than the sample), and falsely high volumes in cases with low values, the degree of error being possibly 20 per cent. We (16) have shown that the mixing of dye in the blood stream is greatly prolonged in congestive heart failure, that an error arises which is in the direction of a falsely low volume when the calculation is based on the dye concentration of samples taken before mixing is complete, and that this error may be as large as 20 per cent in severely decompensated cases. The procedure of using a single sample after injection of the dye involves errors due to dilution of dye by lymph, and to variations in the rate of disappearance from the blood stream.

It is obvious that with all these uncontrolled sources of error inherent in the techniques employed in previously reported studies, two of the most significant of which tend to give falsely low values in subjects with large plasma volumes and

slowed circulation, the finding of low volumes during failure with increases during compensation is an erroneous one.

### CONCLUSIONS

1. In heart disease the change from the compensated to the decompensated state is accompanied by a progressive increase in the volume of plasma and red cells.

2. This increase is shared to a slightly less extent by the plasma than by the corpuscles, resulting in a slight concentration of the blood.

3. The average degree of increase in blood volume above normal parallels the average degree of elevation of venous pressure and slowing of circulation time.

4. During recovery from congestive failure there is a diminution in both plasma and cell volume, the degree of decrease in plasma in most cases being at first more rapid than that of the corpuscles, resulting in varying degrees of blood concentration. With continued compensation the proportion of cells to plasma returns to within normal limits. The decrease in total volume parallels the degree of clinical improvement.

5. In no case was an increase in volume during recovery from chronic congestive failure observed. Relapses to more severe degrees of circulatory failure are accompanied by maintained elevation of, or further increases in blood volume.

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# THE MEASUREMENT OF GLOMERULAR FILTRATION. CREATININE, SUCROSE AND UREA CLEARANCES IN SUBJECTS WITHOUT RENAL DISEASE<sup>1</sup>

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The hypothesis advanced by Rehberg in 1926 (18) that the clearance of creatinine is identical with the glomerular filtration rate in man has stimulated much experimental work in the past decade. But in spite of a considerable body of confirmatory evidence in support of Rehberg's original position, it cannot be said that the actual identity of creatinine clearance with glomerular filtration has been established in man. Chasis, Jolliffe, and Smith (1), Jolliffe and Chasis (9), and Shannon (24) have brought forward certain evidence which they interpret as indicating partial renal secretion of creatinine in man. The studies reported here are concerned with simultaneous creatinine, urea, and (in some cases) sucrose clearances determined in successive consecutive periods. Sucrose was chosen as a substance for comparison with creatinine because its behavior is fairly typical of the whole group of non-metabolized sugars investigated by Smith and Shannon and their associates (1, 9, 10, 11, 27). It may be safely injected intravenously, is readily measured, and is quantitatively recoverable in the urine (12, 13). Urea was included because its behavior has been more widely studied than that of any other so-called "clearance" substance.

## MATERIALS AND METHODS

In this study are included only subjects with creatinine clearances in the "normal" range—i.e., greater than 100 cc. per minute. The subjects were normal volunteers and patients with a variety of clinical conditions not directly affecting the kidneys. No attempt was made to standardize the experimental procedure too rigidly, since this would in part have defeated the purpose of the project, viz., to study the variations in the clearances. In

general the procedure was as follows: At seven o'clock on the day of the experiment the subject was given a single dose of creatinine (10 or 15 grams) and 400 cc. of water. From 30 to 60 minutes later 25 or 30 grams of sucrose were given intravenously in 100 cc. of water. The first urine, taken between 30 and 90 minutes after the creatinine, was discarded. A blood specimen was then taken, followed at intervals by more specimens of blood and urine until the close of the experiment. Large amounts of water were usually taken during the first hour of the experiment and sometimes even later, so that most of the experiments include a considerable period of water diuresis. When the creatinine was administered intravenously, the collection of urine was started about 30 minutes later. In all the sucrose experiments, the first sucrose period was started at about this same interval after the injection of the sucrose. Exact time and divisions of periods are indicated in the table summarizing the clearances.

No attempt was made to synchronize precisely urine collections and blood specimens, save that in general one blood specimen was secured at the beginning of the first urine period, one at the end of the last urine period, and one or more to correspond to each intervening urine period.

The clearances themselves were calculated by extrapolation. In the actual determination of clearances an instantaneously determined quantity (serum concentration) must be compared with an average quantity (average excretion rate during a definite period). In defining a method applicable to all substances at all serum concentrations an entirely general and self-consistent mode of approach must be employed. The method used here is as follows (Figure 1). The various individual serum concentrations were first plotted against time (asterisks), and a smooth curve  $EFF'...$  was drawn through the points. The urine excretion rate was next plotted as a series of straight lines  $BC, B'C', \dots$ . The area under the serum concentration curve corresponding to each urine period ( $AEFD, DFF'D', \dots$ ) was then measured accurately. Each area divided by its abscissa is mathematically the mean value of the serum concentration corresponding to that particular urine collection interval. The clearance was then calculated as urine excretion rate divided by this mean serum figure. The mean clearance thus determined must be distinguished from the instantaneous clearance, which is defined not over a period but rather at a given instant (instantaneous excre-

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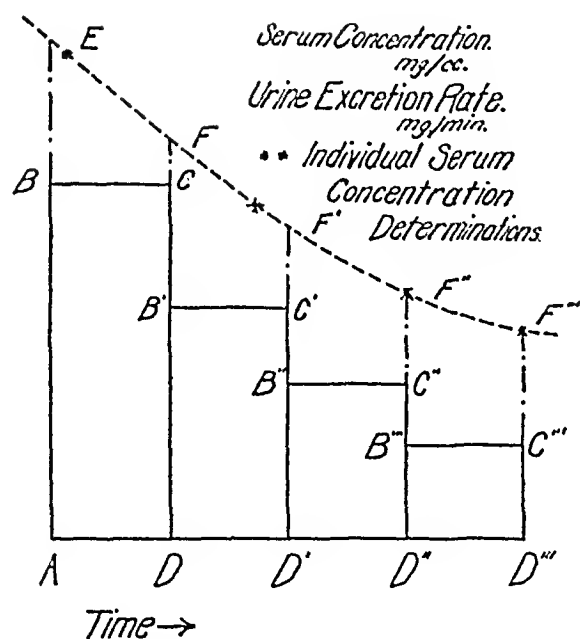


FIG. 1. METHOD OF CALCULATING CLEARANCES

tion rate:serum concentration). The limit of the mean clearance equals the instantaneous clearance as the time

interval during which the urine is collected approaches zero. Any analysis less precise than the foregoing is bound to lead to inconsistencies.

All analyses were made on Folin-Wu tungstic acid filtrates of serum. Creatinine was determined by a slight modification of the original method of Folin (6, 7), sucrose as the difference between the concentration of reducing substance before and after acid hydrolysis, measured by the method of Shaffer and Somogyi (21). Serum sucrose values less than 25 to 30 mgm. per cent were rejected. Urea in earlier experiments was determined by the aeration method of Van Slyke and Cullen (31), later by the gasometric urease method of Van Slyke (29). These gave substantially the same values for clearances (32). Earlier a simple colorimeter, later a Hastings compensating colorimeter, was employed in the estimation of creatinine; with suitable standards good agreement between the results with the two instruments was obtained. Accuracy in measurement of serum creatinine was greater with high serum creatinine levels; as will be seen from Table I, observations with serum creatinine less than 5 mgm. per cent were excluded and the readings were made preferably in the range of 10 to 20 mgm. per cent.

TABLE I †  
Subjects without renal disease

Num- ber	Nature of case	Period	Dose C	Time	Urine	Concentration in serum			Clearance			Clearance ratio		
						C	S	U	C	S	U	C/S	S/U	C/U
			grams	minutes	cc. per minute	mgm. per cent	mgm. per cent	mgm. per cent	cc. per minute	cc. per minute	cc. per minute			
I	Hyperthyroidism M., 39 years, 68 kgm.	1	10	95	2.3	13.0	139	14.0	254	100	21	2.5	4.8	12.1
		2		139	3.8	13.8	85	13.0	216	110	54	2.0	2.0	4.0
		3		182	9.7	12.0	44	13.0	189	128	102	1.5	1.3	1.9
		4		226	3.7	10.2	27	12.8	154	120	52	1.3	2.3	3.0
		5		289	2.1	8.6	19	13.0	200	136	71	1.5	1.9	2.8
II	Normal F., 35 years, 50 kgm.	1	10	110	2.9	10.1	133	11.0	150	126	46	1.2	2.7	3.3
		2		147	8.0	8.0	81	11.0	189	141	60	1.3	2.4	3.2
		3		178	12.1	6.9	58	10.9	136	108	49	1.3	2.2	2.8
		4		207	12.3	6.1	40	11.4	146	111	50	1.3	2.2	2.9
III	Chronic arthritis M., 29 years, 58 kgm.	1	15	100	3.2	16.8	162	9.5	235	143	45	1.6	3.2	5.9
		2		140	4.3	15.0	94	9.1	152	128	81	1.2	1.6	2.0
		3		168	5.0	12.8	62	9.1	121	122	63	1.0	1.9	2.0
IV	Hyperthyroidism, auricular fibrillation M., 43 years, 81 kgm.	1	15	120	1.2	10.8	72	16.7	132	114	44	1.2	3.0	2.6
		2		154	2.3	9.8	54	16.0	278	259	95	1.1	2.6	2.7
		3		192	1.0	8.3	33	15.2	149	126	57	1.2	2.6	2.2
V	Normal M., 26 years, 88 kgm.	1	10	31	10.7	14.5		11.6	226		74			3.1
		2		48	9.1	15.0		13.0	203		60			3.4
		3		69	9.9	12.5		13.0	209		50			4.2
		4		94	10.7	10.2		11.2	181		66			2.7
		5		114	12.3	9.4		10.9	179		72			2.5
		6		145	11.4	7.8		10.5	181		83			2.2
		7		156	8.6	6.8		10.3	170		85			2.0
		8		198	13.9	4.8		12.5	276		102			2.7
		9		260	2.1	4.0		17.2	191		52			3.7
VI	Normal F., 32 years, 70 kgm.	1	10	134	2.1	17.0		10.0	187		52			3.6
		2		232	1.3	13.4		*	169		*			*
		3		305	2.5	10.6		20.0	129		52			2.5
		4		460	0.7	8.2		16.0	96		41			2.3

TABLE I—Continued

Number	Nature of case	Period	Dose C	Time	Urine	Concentration in serum			Clearance*			Clearance ratio		
						C	S	U	C	S	U	C/S	S/U	C/U
			grams	minutes	cc. per minute	mgm. per cent	mgm. per cent	mgm. per cent	cc. per minute	cc. per minute	cc. per minute			
VII	Rheumatic heart disease M., 20 years, 60 kgm.	1	10	107	3.8	13.6		11.7	184		83			2.2
		2		154	3.9	12.0		*	183		*			*
		3		198	10.8	10.8		11.2	178		127			1.4
		4		245	7.0	9.8		13.0	157		61			2.6
		5		302	1.6	8.6		12.8	145		63			2.3
VIII	Obstructive jaundice M., 50 years, 55 kgm.	1	10	153	0.6	14.3		*	139		*			*
		2		203	0.7	11.2		38.6	149		31			4.8
		3		247	0.8	9.4		39.5	143		38			3.8
		4		317	0.8	8.0		32.1	155		38			4.1
IX	Cirrhosis of liver M., 39 years, 67 kgm.	1	10	53	1.7	16.6	75	14.2	221	156	85	1.4	1.9	2.6
		2		97	1.2	13.5	42	13.0	186	149	84	1.3	1.8	2.2
		3		141	1.1	10.4	28	12.5	185	150	84	1.2	1.8	2.2
X	Diabetes mellitus M., 31 years, 57 kgm.	1	10	54	2.4	24.2	90	11.6	197	180	82	1.1	2.2	2.4
		2		104	1.5	15.0	61	11.7	188	150	76	1.3	2.0	2.5
		3		171	1.0	9.5	33	12.6	218	162	66	1.3	2.5	3.3
XI	Diabetes mellitus M., 47 years, 71 kgm.	1	10	53	4.0	25.5	135	16.1	206	108	100	1.9	1.1	2.1
		2		89	3.7	18.0	93	15.0	167	137	93	1.2	1.5	1.8
		3		120	2.4	14.0	73	14.6	165	107	80	1.5	1.3	2.1
		4		160	2.5	10.8	55	14.8	183	121	88	1.5	1.4	2.1
XII	Hyperthyroidism F., 21 years, 50 kgm.	1	10	54	2.8	26.0	150	11.5	168	139	65	1.2	1.4	2.6
		2		94	3.4	18.5	78	9.4	217	208	94	1.0	2.2	2.3
		3		135	4.8	14.5	46	9.2	210	207	103	1.0	2.0	2.0
XIII	Chronic arthritis F., 38 years, 59 kgm.	1	9	60	7.1	26.0		7.2	204		76			2.7
		2		105	4.9	18.0		6.6	169		78			2.2
		3		156	2.6	12.5		6.6	205		70			2.9
XIV	Normal M., 25 years, 75 kgm.	1	10*	53	1.9	17.0		17.0	215		93			2.3
		2		89	2.0	12.4		15.9	171		72			2.4
		3		129	8.0	10.1		15.5	147		90			1.6
		4		180	5.5	7.6		15.0	141		75			1.9
		5		235	2.1	6.0		13.7	154		77			2.0
		6		308	1.2	5.0		12.8	139		74			1.9
		7		390	1.3	4.3		11.9	149		83			1.8

\* Urea injected intravenously.

† C stands for creatinine, S for sucrose, and U for urea nitrogen. All times are calculated to the middle of the period, and all serum concentration figures are extrapolated values. Italic type indicates that the dose of creatinine was administered intravenously.

## RESULTS

The results of 14 such experiments are presented in Table I. They may be analyzed in several ways.

(a) *Variability of the individual clearances.* All three of the clearances vary markedly in successive periods in the same individual. There is a definite tendency in 8 of the 14 cases, Experiments I, III, V, VI, VII, IX, XI, XIV, for creatinine clearances to be high in the earlier periods of the experiments and to decline in successive periods thereafter. Of the remainder, four, Experiments II, IV, X, XIII, are steady or irregular, while only two, Experiments VIII, XII, show any tendency to rise. Neither the sucrose

nor the urea clearance exhibits any such tendency toward systematic decline, although both vary considerably from period to period. The sucrose clearances are the most stable of the three. The urea clearances are apparently the most variable, but without systematic trend, save with urine flow. The relation to urine flow is considered below.

By the exclusion of creatinine clearances below 100 cc. per minute a somewhat arbitrary lower limit has been adopted. It is clear, however, that in subjects with "normal" kidneys the creatinine clearance may range anywhere from 100 to 250 cc. per minute, and that a proportionately wide range exists for the "normal" clearance limits of the other two substances.

(b) *Relation of the clearances to one another.* All of these clearances tend to vary together. In other words, the ratios of the clearances to one another are on the whole less variable than the clearances themselves. However, the creatinine clearance, unlike the other two, tends to decline with time. One aspect of this phenomenon is shown in Figure 2, in which the ratio of creatinine:sucrose clearance is plotted against time.

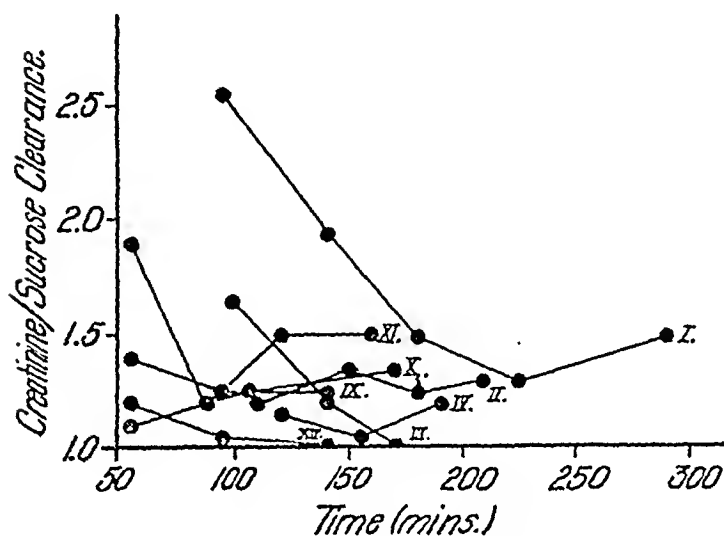


FIG. 2. VARIATION OF CREATININE:SUCROSE RATIO WITH TIME

In three of the eight experiments the ratio drops sharply with time, while in two others it falls off moderately. In the other three there is little change. The creatinine:urea ratio is more variable, reflecting the greater variability of the urea clearance (Figure 3), but here also the declining tendency of the ratio is noticeable. It seems quite clear that the creatinine clearance declines not only in absolute amount but in relation to the sucrose and urea clearances as well.

In no case is there a departure from the sequence of magnitude, creatinine > sucrose > urea, in spite of the individual variation in the clearances. The creatinine:urea ratio varies from a maximum of 12.1 to a minimum of 1.4; but omitting the two highest values which are associated with very low urea clearances and very low urine flow, the upper limit is reduced to 4.0. Within this range all values are found, most of them falling between 2.0 and 3.0. Sucrose clearances in general are somewhat closer to the creatinine than to the urea clearance, the absolute value of the creatinine:sucrose ratio varying from 2.5 to 1.0. The absolute value of the sucrose:urea

ratio varies between 4.8 and 1.1, but the omission of the same pair of low urea clearances reduces the upper limit to 2.7.

(c) *Variation of clearances with urine flow.* This is most marked in the case of the urea clearance, at high (greater than 2 cc. per minute) rates of urine flow as well as at low. No evidence of any definite augmentation limit appears from these data. Thus in Experiment I the urea clearance rose as urine flow increased from 3.8 to 9.7 cc. per minute, only to fall back to its previous value when the urine flow dropped back to 3.7 cc. per minute. In other instances, as in Experiment II, a phenomenon similar to that observed by Shannon (26) in the dog is seen. As the urine flow increased from 2.9 to 8.0 cc. per minute the urea clearance rose, only to drop to its former value with further increase in urine flow. A rise of urea clearance with rising urine flow and subsequent fall with decreasing urine flow occurred in Experiments VII and XIV, and in the last three periods of Experiment V.

It is harder to make definite statements concerning the creatinine and sucrose clearances, especially since the fluctuations of the former with time tend to outweigh other factors. The drop of the creatinine clearance between Periods 8 and 9 of Experiment V may be taken as an instance when variation of urine flow was associated with similar variation of both the creatinine and the urea clearances, while in the first four periods of Experiment I, sucrose and urea clearances varied directionally with urine flow. Other apparent examples of this sort may be picked out. It is, however, quite clear that the tendency of the creatinine clearance to fall off with time cannot usually be correlated with falling rates of urine flow. Whatever tendency the creatinine and sucrose clearances have to vary with urine flow is much less consistent and less marked than is the case with urea.

The sucrose clearance behaves with respect to variation in urine flow like the creatinine rather than the urea clearance. In other words it does not fall off with very low volumes as consistently as does the urea clearance (30).

(d) *Variation of clearances with serum concentration.* The sucrose and the urea clearances do not vary systematically with changes in the concentrations of sucrose and urea in serum re-

spectively. The falling of the creatinine clearances with time is in general directly correlated with a decline in serum creatinine concentration. Since a single large dose of creatinine was given a short period before the commencement of the experiments, and not repeated later, it was inevitable that the serum creatinine be in a declining

declined. The existence of so many exceptions to the correlation is strong evidence that the association is accidental, due to the mode of planning of many of the experiments.

(e) *Intravenous and peroral administration.* Creatinine was given orally in 8 subjects and intravenously in 6. The peculiar behavior of the

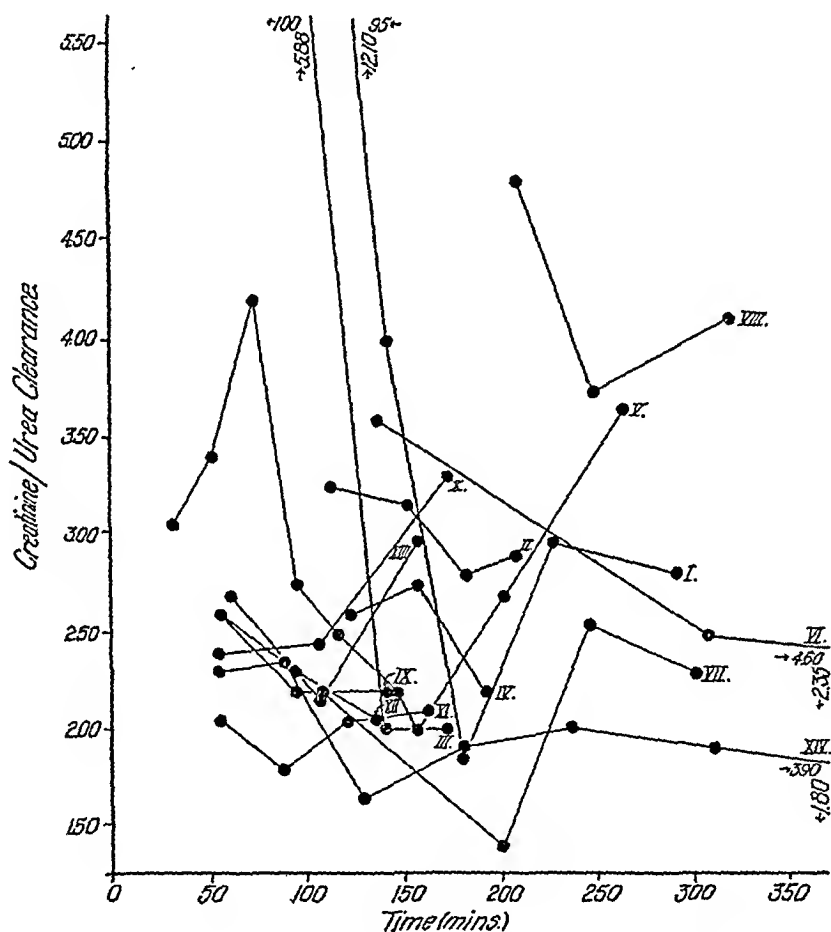


FIG. 3. VARIATION OF CREATININE: UREA RATIO WITH TIME

phase throughout the greater part of the experiments, irrespective of the mode of variations of the clearances. The fortuitous nature of the correlation is indicated by the existence of certain instances in which the creatinine clearances fell off while the serum concentration was still rising slightly (Experiment I, Periods 1 to 2; Experiment V, Periods 1 to 2). In Experiments II and X the serum creatinine fell off while the clearances did not, and in Experiment XII the clearance actually rose while the serum concentration

creatinine clearance is equally apparent in both groups. There is no evidence from the experiments that the administration of either creatinine or of sucrose intravenously in the concentrations and amounts used had any appreciable effect on the clearances themselves or on urine flow. In the 4 experiments in which small amounts of urea were injected simultaneously with the creatinine, in one instance only (Experiment VII, Period 3) did the urea clearance rise following the injection.

(f) *Type of case.* No definite inference can



be drawn because of the limited number of conditions represented. The three patients with hyperthyroidism (Experiments I, IV, XII) had rather high clearances with more than the average degree of variability. This is consistent with the observations of others (15).

#### DISCUSSION

If two entirely different substances have numerically identical clearance values, a simple explanation for such identity may be found in their common excretion by filtration alone. Any other explanation would be difficult and arbitrary, especially in the face of all the collateral evidence demonstrating the reality of glomerular filtration. Three or more substances having identical clearances would still further increase the improbability of any alternative explanation. It is essential that the substances differ both chemically and physiologically; for example, identity of clearances of several foreign sugars is not nearly such cogent evidence.

In the dog, both the creatinine and the inulin clearances are identical (19, 23, 25). Also, Van Slyke, Hiller and Miller (33) have recently shown that the ferrocyanide clearance is identical with the clearances of these other two. If the general theory of filtration and reabsorption is accepted, then certainly the burden of proof rests on the shoulders of anyone who would deny that this common clearance of creatinine, inulin, and ferrocyanide represents the actual glomerular filtration rate in the dog. Unfortunately, no such identity is demonstrable in man. Creatinine clearance in man is definitely somewhat higher than that of inulin (24) and much higher than that of ferrocyanide (17). By analogy with the dog it may reasonably be supposed that the glomerular filtration rate is somewhat in the neighborhood of the inulin and creatinine clearances, but the assertion that one or the other of these substances represents the true filtration rate is at present distinctly arbitrary.

At first glance creatinine would seem to be more likely than inulin, because its clearance is somewhat the larger. However, Shannon (24) bases his belief that inulin is simply filtered and creatinine both filtered and secreted, partly on the behavior of the creatinine clearance after phlori-

zin (1, 28), but more especially on the peculiar behavior of the creatinine: inulin ratio after the intravenous injection of large amounts of creatinine. At the start of these experiments with the serum creatinine at 10 to 20 mgm. per cent the creatinine:inulin ratio was about 1.4. Repeated injections of creatinine were given so that the serum creatinine level rose to about 100 mgm. per cent, and simultaneously the creatinine:inulin ratio fell to about 1.1 and remained there. This fall in ratio was accomplished almost entirely through a drop in the creatinine clearance, the absolute value of the inulin clearance changing but little. However, as the serum creatinine dropped gradually back to its initial level, the creatinine:inulin ratio remained at 1.1 instead of rising to 1.4 again. Shannon attempts no explanation of this latter effect, but concludes that creatinine clearance varies with serum concentration, and that consequently, in man, the inulin clearance is a truer measure of glomerular filtration than is the creatinine clearance.

In a considerable proportion of experiments here reported the creatinine clearance fell in the successive periods after the administration of the substance both absolutely and relative to the sucrose clearance. Since sucrose behaves as does xylose (10, 11, 12), yielding clearances constantly 20 or 25 per cent less than inulin, these experiments may be related to those of Shannon just mentioned. In both sets of experiments the initially high creatinine:sugar ratio gradually declined during successive periods, and in both sets this drop was brought about through a decline in absolute value of the creatinine clearance, the sugar clearance remaining practically constant. In Shannon's experiments the decline in ratio is partly associated with rising concentration of creatinine in the serum, while in ours the correlation is, if anything, with a declining serum concentration. The emphasis which Shannon lays on the correlation between absolute serum creatinine concentration and creatinine:inulin ratio is, however, not justified by his own data, since in the later stages of his experiments the serum concentration of creatinine declines from higher to much lower levels without change in the ratio. It appears as though the correlation in his experiments between rising serum concentration and declining ratio were fortuitous, depending on the manner in



which the experiments were performed. Certainly our results are entirely consistent with those of Shannon; only the common factor with which the drop in the creatinine clearance and the creatinine:sugar ratio may be correlated is not serum creatinine concentration, but time after injection or ingestion. If it be assumed that for some reason creatinine is eliminated at an abnormally high rate after a large exogenous dose, and then at a more usual rate in later periods, the two sets of experiments are entirely consistent.

Abnormally high clearances immediately after giving creatinine have certainly been observed before. The protocols of Chrometzka and Unger (2), of Iverson and Jacobsen (8) and of Medes and Berglund (14) offer examples of this effect. Rehberg's original protocols show the effect to a slight degree (18) as do those of Dominguez and Pomerene (5). Indeed, these latter authors base their major contention on this observation, but interpret it as a minor artificial perturbation due to the inclusion of "endogenous" with "exogenous" creatinine of serum and urine in the usual calculation of clearance. They show that if the clearances are "corrected" by subtracting from the creatinine of serum and urine the endogenous fraction, the progressive decline of values is eliminated. They conclude therefrom that endogenous and exogenous creatinine are excreted by different mechanisms. At the levels of serum and urine creatinine in most of our experiments such endogenous "corrections" are quantitatively very small and certainly fail entirely to bring successive clearances into line. It seems probable that Dominguez and Pomerene were observing a phenomenon similar to that here reported, but were misled by the accidental coincidence of the magnitude of the necessary mathematical adjustment with the normal values of endogenous creatinine. It should be noted that their doses of creatinine as well as those of Rehberg were one-half to one-third as great as ours. Similar considerations apply to the proposed correction of Cope (3), which was to be applied to the serum creatinine values alone.

Some caution is necessary before interpreting these irregular fluctuations in the creatinine clearance as "tubular secretion" over and above glomerular filtration along the lines suggested by

Shannon and Smith. However, an interpretation of these fluctuations as actual changes in glomerular filtration rate makes it very difficult to understand the simultaneous stability of the other clearances. Still another interpretation is possible, viz., that some of the Jaffe reacting substance in the serum is not true creatinine, and that the proportion of this non-creatinine chromogenic substance decreases with time. This is rendered most improbable by the recent demonstration by Miller and Dubos (16) that all the chromogenic substance in normal serum is true creatinine. Clearly an explanation of this type is fanciful; the preponderance of evidence favors tubular secretory activity as the explanation presenting the fewest difficulties.

It cannot be too strongly emphasized that our observations relate only to man. In the dogfish, relatively enormous creatinine:inulin ratios have been observed (22), of an entirely different order of magnitude from those seen in man. This phenomenon may well be due to quite different causes. Some tendency for the creatinine clearance to fall off with time has been observed in dogs by Davenport, Fulton, Van Auken and Parsons (4), and by Schmitz (20). In view of the repeated demonstrations by Shannon and others of the identity of creatinine and inulin clearances in this animal, it is only reasonable to assume that these changes represent actual changes in glomerular filtration rate, and are not comparable to the phenomenon observed by us in man.

The practical implication of our data in the interpretation of single clearances is obvious. Single creatinine clearances, without regard to time after administration, are grossly misleading, if they are assumed to be measures of filtration rate. Urea clearances vary so much with urine flow that all individual clearances must be interpreted with this in mind. And sucrose is presumably reabsorbed to a slight but uncertain extent. Thus, though all these clearances tend to vary together, each is subject to specific perturbing factors. Hence no one of them may be considered an absolute measure of glomerular filtration; yet, subject to the several reservations that have been set forth with each individual substance, they are all relative measures of glomerular filtration.

## CONCLUSIONS

1. Creatinine, sucrose and urea clearances in man, while subject to considerable variation in the same individual, tend to vary together.

2. The order of magnitude of clearances: creatinine > sucrose > urea is rigidly maintained.

3. Creatinine clearances sometimes tend to be high in periods immediately following the administration of the creatinine, but fall off as time goes on.

4. Sucrose and urea clearances measured simultaneously have no such tendency to decline with time.

5. This peculiar behavior of creatinine probably represents varying tubular secretion rather than varying glomerular filtration.

6. Creatinine, sucrose and urea clearances are, subject to certain limitations inherent in each one, satisfactory relative measures of glomerular filtration. None of them is an absolute measure of glomerular filtration.

7. Individual creatinine clearances must be interpreted with caution.

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# THE MEASUREMENT OF GLOMERULAR FILTRATION. THE CREATININE, SUCROSE AND UREA CLEARANCES IN SUBJECTS WITH RENAL DISEASE<sup>1</sup>

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In a previous communication (9) the manner of variation of creatinine, sucrose, and urea clearances was studied in a group of subjects without renal disease. The method involved the determination of these three clearances simultaneously in consecutive time intervals under varying conditions of urine flow. It was concluded from their behavior that change in glomerular filtration was an underlying common factor responsible for their variation, but that each one of the three clearances individually was subject to special perturbing factors.

In this paper these observations are extended to include a group of subjects with conditions affecting the renal status.

## MATERIALS AND METHODS

The subjects of these investigations were patients on the medical wards of the New Haven Hospital. Clearances of creatinine, urea and in many instances sucrose, were determined in two or more consecutive periods. The conduct of the experiments, the methods of chemical analysis, and the mode of calculating clearances are all described in the preceding paper (9).

## RESULTS

### (A) General observations

In Table I are presented the data from 11 patients who, in spite of conditions which may have affected the vascular system or the kidneys, had no significant reduction of their clearances. In Table II are presented the studies of 11 other pa-

tients whose renal or vascular disease was associated with marked reduction of clearances.

In Table I there is no significant difference between the behavior of the clearances of these subjects and the behavior of strictly normal subjects. The creatinine clearance declines with time, both absolutely and relative to the sucrose clearance, in Experiments I, II, III, IV and VII (Figure 1A). The variation of the creatinine:

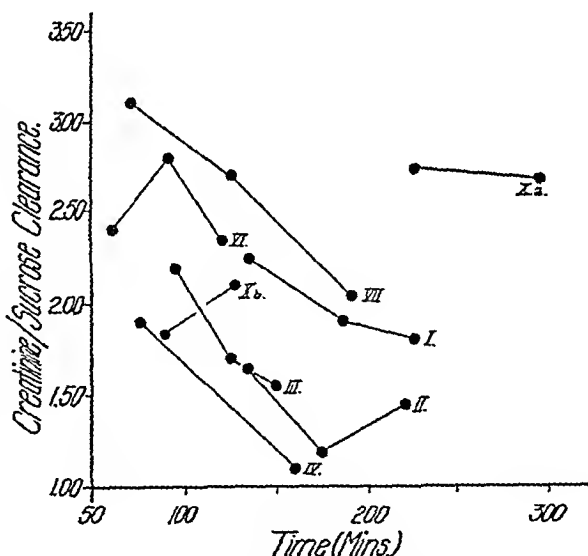


FIG. 1A. VARIATION OF CREATININE: SUCROSE RATIO WITH TIME IN SUBJECTS WITHOUT DEPRESSION OF CLEARANCES

sucrose ratio is irregular in Experiments VI, Xa and Xb. The variations of the creatinine:urea ratio (Figure 1B) are less consistent. The ratio declines with time in Experiments I, IV, VIII, IX and XI, and shows no significant change in Experiments II, VI, Xa and Xb. In Experiments III, V and VII the creatinine:urea ratio actually rises. During the course of the three experiments in which the creatinine:urea ratio rises the urea clearance itself declines and the

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# THE MEASUREMENT OF GLOMERULAR FILTRATION. THE CREATININE, SUCROSE AND UREA CLEARANCES IN SUBJECTS WITH RENAL DISEASE<sup>1</sup>

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In a previous communication (9) the manner of variation of creatinine, sucrose, and urea clearances was studied in a group of subjects without renal disease. The method involved the determination of these three clearances simultaneously in consecutive time intervals under varying conditions of urine flow. It was concluded from their behavior that change in glomerular filtration was an underlying common factor responsible for their variation, but that each one of the three clearances individually was subject to special perturbing factors.

In this paper these observations are extended to include a group of subjects with conditions affecting the renal status.

## MATERIALS AND METHODS

The subjects of these investigations were patients on the medical wards of the New Haven Hospital. Clearances of creatinine, urea and in many instances sucrose, were determined in two or more consecutive periods. The conduct of the experiments, the methods of chemical analysis, and the mode of calculating clearances are all described in the preceding paper (9).

## RESULTS

### (A) General observations

In Table I are presented the data from 11 patients who, in spite of conditions which may have affected the vascular system or the kidneys, had no significant reduction of their clearances. In Table II are presented the studies of 11 other pa-

tients whose renal or vascular disease was associated with marked reduction of clearances.

In Table I there is no significant difference between the behavior of the clearances of these subjects and the behavior of strictly normal subjects. The creatinine clearance declines with time, both absolutely and relative to the sucrose clearance, in Experiments I, II, III, IV and VII (Figure 1A). The variation of the creatinine:

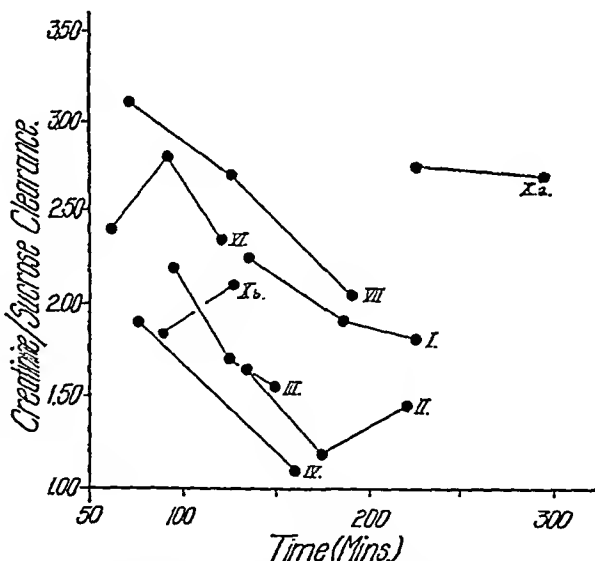


FIG. 1A. VARIATION OF CREATININE: SUCROSE RATIO WITH TIME IN SUBJECTS WITHOUT DEPRESSION OF CLEARANCES

sucrose ratio is irregular in Experiments VI, Xa and Xb. The variations of the creatinine:urea ratio (Figure 1B) are less consistent. The ratio declines with time in Experiments I, IV, VIII, IX and XI, and shows no significant change in Experiments II, VI, Xa and Xb. In Experiments III, V and VII the creatinine:urea ratio actually rises. During the course of the three experiments in which the creatinine:urea ratio rises the urea clearance itself declines and the

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TABLE I†

*Subjects with diseases affecting the kidneys but with creatinine clearances greater than 80 cc. per minute*

Num- ber	Nature of case	Period	Dose C	Time	Urine	Concentration in serum			Clearance			Clearance ratio		
						C	S	U	C	S	U	C/S	S/U	C/U
			grams	minutes	cc. per minute	mgm. per cent	mgm. per cent	mgm. per cent	cc. per minute	cc. per minute	cc. per minute			
I	Toxemia of pregnancy, de- livered; hypertension F., 32 years, 88 kgm.	1	15	134	2.5	14.0	143	12.5	248	111	46	2.2	2.4	5.4
		2		184	1.8	10.8	97	12.5	209	110	45	1.9	2.6	4.7
		3		227	4.3	9.5	67	12.3	197	108	45	1.8	2.4	4.4
II	Progressive muscular atrophy, arteriosclerosis M., 51 years, 63 kgm.	1	15	135	2.6	18.4	140	18.8	153	92	49	1.7	1.9	3.1
		2		175	2.1	17.1	106	18.7	109	82	38	1.2	2.2	2.9
		3		220	2.6	16.1	79	18.6	100	68	33	1.5	2.1	3.0
III	Nephrotic syndrome F., 16 years, 60 kgm.	1	10	95	3.9	13.1	150	7.8	221	81	113	2.2	0.7	2.0
		2		124	1.8	14.0	112	7.6	122	58	48	1.7	1.2	2.5
		3		152	2.2	14.0	86	7.6	154	50	52	1.5	1.5	3.0
IV	Nephrotic syndrome F., 16 years, 80 kgm.	1	10	75	3.4	12.3	75	23.4	185	98	49	1.9	2.0	3.8
		2		160	1.3	12.6	42	22.8	100	91	33	1.1	2.8	3.0
V	Arteriosclerosis M., 41 years, 75 kgm.	1	10	119	5.1	15.5		5.9	98		62			1.6
		2		163	2.9	14.1		5.9	109		64			1.7
		3		223	1.5	13.2		6.8	109		49			2.2
		4		281	1.2	12.2		8.0	110		41			2.7
		5		403	0.7	9.6		9.7	93		29			3.2
VI	Acute glomerulonephritis, subsiding M., 15 years, 65 kgm.	1	5	59	4.5	9.0	100	9.0	264	111	114	2.4	1.0	2.3
		2		91	5.1	6.6	62	9.1	256	91	107	2.8	0.9	2.4
		3		121	3.6	5.4	52	8.8	224	95	86	2.4	1.1	2.6
VII	Arteriosclerosis, diabetes mellitus M., 62 years, 67 kgm.	1	5	68	1.8	9.4	100	12.7	219	71	56	3.1	1.3	3.9
		2		123	0.7	6.5	65	13.4	133	49	32	2.7	1.5	4.2
		3		188	0.6	5.3	40	13.8	147	71	29	2.1	2.5	5.1
VIII	Diabetes insipidus M., 21 years, 63 kgm.	1	10	20	16.8	28.6		8.0	211		86			2.5
		2		50	20.0	21.7		7.0	167		127			1.3
		3		73	26.1	18.8		6.5	200		159			1.3
		4		95	12.8	16.0		7.6	129		77			1.7
IX	Acute focal nephritis M., 23 years, 74 kgm.	1	10	157	2.2	17.9		16.3	214		55			3.9
		2		193	5.7	15.5		15.7	202		69			2.9
		3		225	8.5	13.5		15.5	197		72			2.7
X	Acute glomerulonephritis M., 16 years, 75 kgm.	1†	10	195	2.8	8.7	77	25.0	235	85	46	2.8	1.9	5.1
		2		300	2.4	7.1	66	26.0	211	78	40	2.7	2.1	5.3
		1¶	10	70	3.2	13.9	147	21.9	109	60	47	1.8	1.3	2.3
		2		127	3.3	15.5	117	21.9	141	67	57	2.1	1.2	2.5
XI	Nephrosclerosis M., 60 years, 56 kgm.	1	10	168	12.7	10.9		15.2	100		20			5.0
		2		211	17.2	10.2		*	169		*			*
		3		245	10.0	9.2		21.0	108		32			3.4
		4		298	8.2	8.5		20.3	97		28			3.5
		5		367	6.7	8.2		20.3	82		30			2.8

\* Urea injected intravenously.

† C stands for creatinine, S for sucrose, U for urea nitrogen. All times are calculated to the middle of the period, and all serum concentration figures are extrapolated values. *Italic type indicates that the dose of creatinine was administered intravenously.*

‡ January 6.

¶ February 10.





TABLE II—Continued

Number	Nature of case	Date	Period	Dose C	Time	Urine	Concentration in serum			Clearance			Clearance ratio		
							C	S	U	C	S	U	C/S	S/U	C/U
				grams	minutes	cc. per minute	mgm. per cent	mgm. per cent	mgm. per cent	cc. per minute	cc. per minute	cc. per minute			
VI	Mercuric bichloride poisoning F., 46 years, 86 kgm.		1	15		0.1	9.2		104	0.7		0.3			2.3
			2			0.1	9.7	438	108	0.4	0.4	0.2	1.0	2.0	2.0
VII	Acute glomerulonephritis M., 13 years, 42 kgm.		1	(16)†	116	2.6	35.4	228	26.7	59	39	26	1.5	1.5	2.3
			2		150	1.4	30.5	198	28.0	45	25	16	1.8	1.6	2.8
			3		176	1.3	29.5	186	29.0	36	23	15	1.6	1.5	2.2
VIII	Chronic glomerulonephritis, terminal M., 54 years, 62 kgm.	May 12	1	8	78	0.7	22.4	77	77.2	6.6	6.1	3.6	1.1	1.7	1.9
			2		134	0.7	21.4	77	77.1	7.2	6.1	4.0	1.2	1.5	1.8
			3		175	0.8	18.3	73	77.1	8.7	5.9	4.5	1.5	1.3	1.9
		May 31	4		228	0.9	15.8	69	77.4	9.6	6.5	7.8	1.5	0.8	1.2
			1		80	0.4	23.5	40	65.0	3.0	3.2	2.3	1.0	1.4	1.3
IX	Nephrosclerosis F., 45 years, 103 kgm.		1	15	111	3.5	23.8	143	20.4	100	66	43	1.5	1.5	2.3
			2		208	0.9	20.7	133	19.8	49	32	20	1.5	1.6	2.5
X	Nephrosclerosis‡ rheumatic heart disease M., 56 years, 54 kgm.		1	10	45	2.2	38.5		84.3	21.7		9.8			2.2
			2		81	1.8	32.8		84.0	19.1		9.7			2.0
			3		115	1.7	28.5		84.7	21.6		10.2			2.1
			4		162	1.0	27.0		83.3	15.0		6.9			2.2
			5		228	1.4	26.0		83.7	14.8		8.1			1.8
			6		315	1.7	24.2		83.9	17.8		10.3			1.7
XI	Acute glomerulonephritis M., 33 years, 98 kgm.		1	10	Given previous night	1.0	5.7		56.2	22.8		5.6			4.1
			2			1.5	5.7	360	56.2	20.5	17.8	7.1	1.2	2.5	2.9
			3			1.2	5.7	330	56.2	17.7	15.7	5.8	1.1	2.7	3.1
			4			0.8	5.7	320	57.3	15.0	12.1	3.3	1.3	3.7	4.6
			5			0.7	5.7	320	57.4	14.1	8.5	3.1	1.7	2.7	4.6
			6			0.6	5.7	315	57.4	13.9	8.8	1.9	1.6	4.6	7.3
			7			0.5	5.7	310	57.3	12.7	7.5	1.9	1.7	4.0	6.7

† C stands for creatinine, S for sucrose, and U for urea nitrogen. All times are calculated to the middle of the period, and all serum concentration figures are extrapolated values. Italic type indicates that the dose of creatinine was administered intravenously.

\* 5.0 grams of urea in normal saline given intravenously in this period.

‡ 8.0 grams of creatinine given per os, followed almost immediately by vomiting; then 8.0 grams given intravenously.

§ It is possible that the nephrosclerosis was secondary to antecedent renal damage, since there was evidence of bilateral hydronephrosis by x-ray.

volume these subjects behave as would subjects with entirely normal renal function. Also, the variation of the clearances together in a fixed order of magnitude is similar to the behavior of clearances in quite normal subjects.

In Table II, representing subjects with some definite reduction of clearances, there is no such decline of the creatinine clearance or of the creatinine:sucrose and creatinine:urea ratios with time (Figures 2A and 2B). There are at least four other outstanding differences apparent in comparing this group with impaired clearances with the subjects of Table I, or with a normal group.

(1) The absolute numerical range of variation of the three clearances in any individual (in cc. per minute) is less than normal.

(2) The relative degree of variability (on a percentage basis) from period to period is much less.

(3) The clearance ratios, creatinine:urea, sucrose:urea, and creatinine:urea, in a given subject are much more constant than in a comparable subject with normal clearance levels.

(4) With marked reduction in the level of the clearances the creatinine:sucrose, sucrose:urea, and creatinine:urea ratios are smaller than usual, i.e., the clearances are nearer together. In two instances, the sucrose and creatinine clearances were identical.

An examination of the data shows that part of this stability of clearance is correlated with considerable stability of urine flow. This is doubtless

an expression of the slow and relatively slight variation in urine flow which the nephritic with serious renal functional impairment shows in response to drinking water or other diuretic measures. However, in these low clearance cases there is some tendency for clearances and urine flow to vary simultaneously, as for example in Cases I and X.

continues to rise. This behavior resembles that of normal subjects.

(B) *The relation of clearances to type of case*

(1) *Nephritis.*

(a) *Acute nephritis (6 cases).* Of these, two (Table I, Cases VI and IX) had recovered clini-

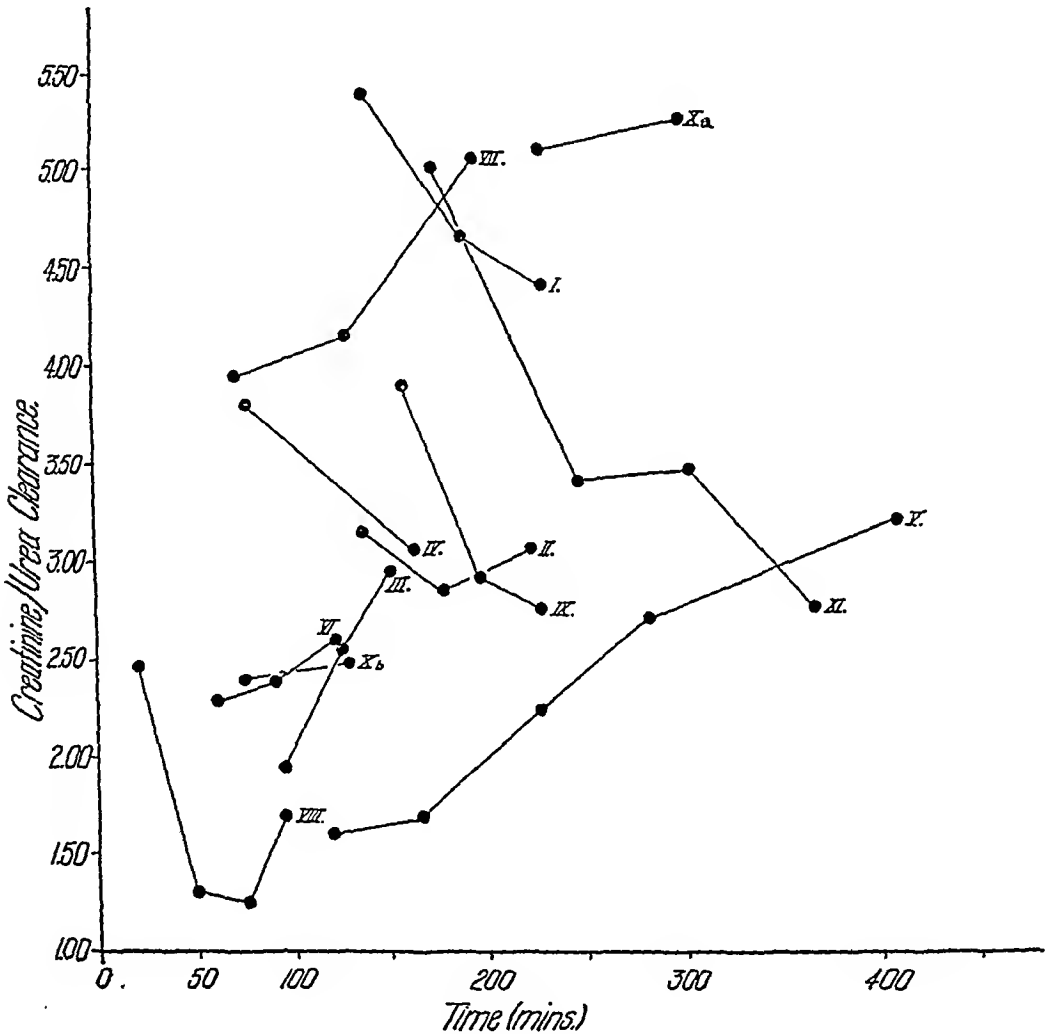


FIG. 1B. VARIATION OF CREATININE:UREA RATIO WITH TIME IN SUBJECTS WITHOUT DEPRESSION OF CLEARANCES

The association of sudden increase in urine flow with transitory rise in the urea clearance is found in certain of the cases with depressed clearances. Thus in Experiment V the urea clearance increases with the rising urine flow between Periods 2 and 3 (July 8), and then falls back to its previous level in Period 4, although the urine flow

cally, and all their clearances in absolute magnitude and in behavior are entirely normal. In one (Table II, Case VII), who later recovered completely after a fairly prolonged course, all clearances were depressed to about one-third normal at the time of this observation, some two weeks or so after the onset. They were entirely normal in

their relations to one another at this time. No observations earlier or later were made. One (Table II, Case XI) went on to a fatal exitus from cerebral hemorrhage. His course was character-

Case X) the initial observations were made within a few days after the onset of the nephritis. The first of these (Table II, Case V) had a very violent and explosive acute nephritis

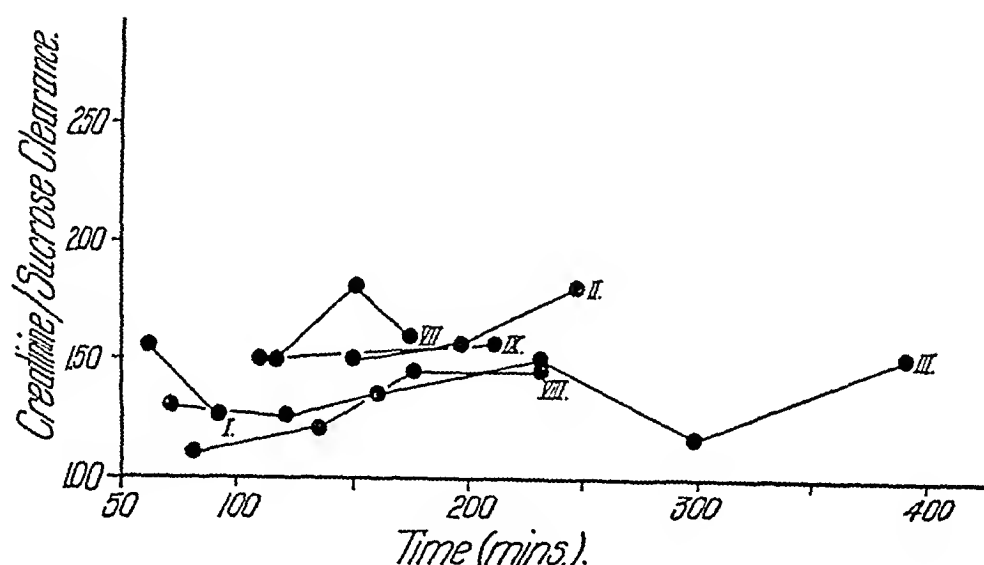


FIG. 2A. VARIATION OF CREATININE:SUCROSE RATIO WITH TIME IN SUBJECTS WITH DEPRESSED CLEARANCES

ized by persistent and progressive oliguria, hypoproteinemia, albuminuria, edema, and elevation of blood nonprotein nitrogen concentration. There is merely a depression of all clearances to about one-tenth of their normal values. In the two remaining cases (Table II, Case V and Table I,

with gross persistent hematuria, albuminuria and retinal hemorrhages. The first observation was made three days after the onset of hematuria. At this time although the serum urea nitrogen was 88 mgm. per cent and the urea clearance 5 cc. per minute, the creatinine clearance was almost

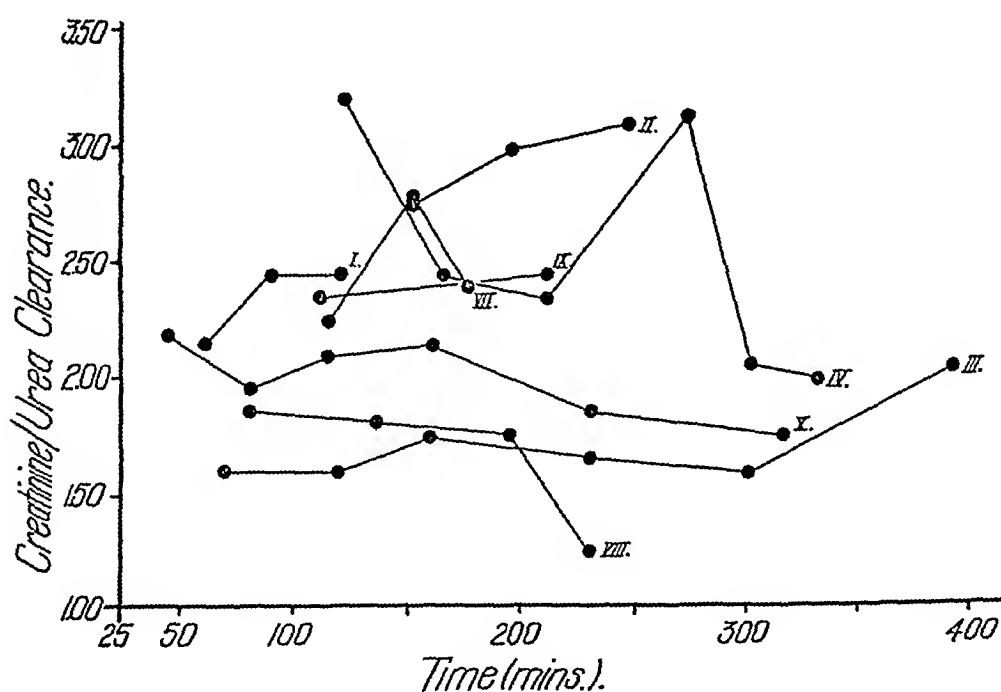


FIG. 2B. VARIATION OF CREATININE:UREA RATIO WITH TIME IN SUBJECTS WITH DEPRESSED CLEARANCES

normal (about 100 cc. per minute). During the next few months the patient improved considerably but did not clear up entirely, and now one year later presents a picture of subacute or chronic active glomerulonephritis. In the month following the initial observation the creatinine clearance fell and the urea clearance rose until they stood in a normal relation to one another but both at depressed levels. The continued depression thereafter is correlated with continuance of the active process. The second case of acute nephritis (Table I, Case X) was first studied about a week or more after the onset of hematuria. The course was much more benign than the one just described; within a few days the hematuria and albuminuria virtually disappeared. At the time of the initial observations, however, creatinine clearance was very high, both absolutely and relative to the sucrose and the urea clearances, and in this respect the reaction is comparable to that of the preceding case. This impression is strengthened by the fact that the repeated determination of the three clearances after subsidence of the acute process shows a lower creatinine:sucrose and a much lower creatinine:urea ratio, both ratios being now quite within the normal range.

(2) *Chronic nephritis (9 cases).*

(a) "Lipoid nephrosis" without vascular disease (2 cases, Table I, Cases III and IV). These were both 16 year old girls who suddenly developed massive proteinuria, with leukocytes and casts in the urine sediment, hypoproteinemia and lipemia, and generalized edema, without hematuria or vascular disease. Clearances were entirely normal in magnitude and in behavior, including the fall of the creatinine clearances with time. These are the only subjects with definite chronic renal disease who had normal clearances.

(b) Chronic nephritis with vascular disease (7 cases, Table II, Cases I, III, IV, VIII, IX, X and Table I, Case XI). These cases present all degrees of reduction of clearances down to one-tenth or even one-twentieth of normal. In all, the clearances and urine flow are relatively fixed and in most the ratios between clearances are lower than normal. The three cases with creatinine clearances less than 20 cc. per minute all died within

a few weeks. The single observation of Case VIII on May 31, 1935, was made a few days before death, and is one of the two observations in which the sucrose and creatinine clearances are identical.

(c) Chronic pyelonephritis (infection with *B. coli*) (1 case, Table II, Case II). This was a case of chronic ascending pyelonephritis in a female subject with probable secondarily contracted kidneys. As in chronic nephritis of the vascular type, the clearances are uniformly depressed and vary but little from period to period.

(3) *Mercury poisoning (1 case, Table II, Case VI).*

These clearances were determined on the ninth day of  $\text{HgCl}_2$  poisoning in a woman who died three days later. After two or three days of initial anuria following the ingestion of the poison the patient began passing very small amounts of urine, without any tendency to increase the volume in the succeeding days. The second clearance was obtained between two catheterizations and is thus exact. It is the lowest clearance in the series, and is the second of the two instances in which the creatinine and sucrose clearances were identical. Even here the urea clearance is definitely lower than the other two.

(4) *Vascular disease without evidence of renal disease (4 cases).*

(a) Generalized arteriosclerosis (3 cases, Table I, Cases II, V, and VII). In two of the three cases the clearances though still in the normal range, are very close to the lower limits of normal. In the remaining case they are entirely normal.

(b) Hypertension following toxemia of pregnancy (1 case, Table I, Case I). In this case the clearances are entirely normal in magnitude and behavior.

(5) *Diabetes insipidus (1 case, Table I, Case VIII).*

While the clearance of creatinine is quite normal, that of urea is supra-normal, reaching at one time 159 cc. per minute, the highest in this series and higher than any true normal. It is associated with the abnormally high urine volume of 26 cc. per minute.

## DISCUSSION

Data secured from such a wide variety of pathological states as are here represented are apt to raise more questions than they answer. All the more remarkable then does it seem that the order of clearances, creatinine  $>$  sucrose  $>$  urea, should have been so regularly maintained with such a range of material and under such varying conditions. In only a certain number of instances were the ratios of the clearances to one another outside the usual range. A peculiarly fundamental physiological significance of these clearances is indicated by the fact that their magnitude alone should be affected in the most varied types of renal damage.

The exceptions to the rule are of peculiar interest. The two instances in which creatinine and sucrose clearances were equal, are also the two with the lowest clearances. Both patients had kidneys which were so badly impaired that death ensued from renal failure within a few days. Even in these cases the urea clearance was appreciably lower than the other two, perhaps because passive back diffusion persisted as long as any filtration whatsoever continued. Cambier (1) in one case of  $\text{HgCl}_2$  poisoning with recovery found the urea and creatinine clearances identical at first. They gradually diverged as the patient recovered until they returned to their normal relationship to one another. The observations in our case were made some days after urine flow had recommenced, and so are not comparable. The tendency of cases with advanced renal disease to have the three clearances closer together than normal is also noteworthy and probably indicates that with increasing renal damage, tubular secretion of creatinine as well as reabsorption of sucrose and urea diminish.

High creatinine and low urea clearances in the early stages of acute nephritis may indicate that tubular secretion of creatinine is increased at this early stage of the disease. An alternative interpretation, persistently high filtration with increased reabsorption of urea, is possible. However, the hypothesis of tubular secretion seems somewhat more probable in view of the fact that the sucrose clearance is aligned with the urea rather than with the creatinine clearance. Furthermore, Goldring (4) has recently demonstrated

that in this same early stage of acute nephritis, the phenol red clearance is disproportionately elevated, again indicating excessive tubular secretory activity under these special circumstances.

The two cases with nephrosis show a quite intact tubular "secretory" mechanism, if the fall of creatinine clearance with time may be so interpreted. This is entirely consistent with the ability of these patients to concentrate urine to a high degree, and to reabsorb salt completely. The absence of any reduction in the magnitude of the clearances, furthermore, indicates an intact glomerular filtration rate; so apparently the nephrotic state might be explained by a slightly increased permeability of the glomerular membrane which allows albumin (but very little globulin and no red cells) to pass by it along with the ultrafiltrable solution. This does not seem consistent with the demonstration that the chief cellular pathology exists in the tubular cells (10), but no ready explanation of this paradox is at present forthcoming.

Taking all these regularities and occasional exceptions together the thesis that all three clearances in general are measures of glomerular filtration is strengthened. Such regular behavior may be accounted for easily on such a basis and upon no other.

The fact that the badly damaged kidneys did not show the peculiar drop of the creatinine clearance with time, whereas those without impairment of clearance behaved like normals, would support the view that this peculiar effect is renal in origin, without pointing to the particular part of the kidney responsible for it.

The significance of this study in elucidating the phenomena of renal damage is chiefly incidental. Our results confirm and supplement the findings of Holten and Rehberg (6, 7), Ellis and Weiss (3), Hayman et al. (5), Cope (2), Cambier (1), and others who have studied creatinine and urea clearances simultaneously and have in general found them impaired to a parallel degree in renal disease. In the light of the filtration interpretation of clearance this must mean that usually in renal disease when glomerular filtration is reduced, reabsorption of urea is correspondingly impaired. The most reasonable interpretation of such a consistent effect is that the number of

glomeruli filtering at any time is reduced in renal disease, but that those glomeruli which are actively filtering are associated with tubule cells which reabsorb nearly their usual percentage of urea. Shannon (8) and others have suggested that in the intact kidney urea is reabsorbed both by passive diffusion and by cellular activity in the tubules. The example of extreme depression of clearances in which the urea clearance is closer than usual to the creatinine could be readily explained with such a dual theory by assuming that with progressive renal damage the active power to reabsorb urea is lost, leaving a certain measure of back diffusion only. Such an interpretation must however needs be quite hypothetical until it can be decided whether two such mechanisms actually exist in the intact kidney. Our observations do not bear on this most fundamental point, so the question of definite interpretation of the lowering of the creatinine:urea clearance ratios in advanced renal disease must await other experience.

#### CONCLUSIONS

1. The behavior of the creatinine, sucrose, and urea clearances in subjects with various renal and vascular diseases differs somewhat from their behavior in normal subjects.
2. In the presence of definite renal disease the absolute magnitude of all three clearances is usually consistently and uniformly reduced, while the normal order of the clearances, creatinine > sucrose > urea is in general maintained.
3. In "nephrosis" all of the clearances may be normal in magnitude and behavior.
4. In the early stages of acute nephritis the creatinine clearance may be disproportionately high.
5. The falling off of the creatinine clearance with time, which is seen in normal subjects, is not to be found in subjects with depressed clearances.
6. In subjects with depressed clearances the

absolute and relative variability of the clearances is reduced, as are the numerical values of the ratios of the clearances to one another.

7. The behavior of these clearances in the presence of renal disease is consistent with the belief that as in the normal subject they are all relative measures of filtration. Their behavior is also consistent with the theory that the degree of reduction of these clearances reflects quantitatively the degree of reduction of the glomerular filtration rate.

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# A CONVENIENT METHOD FOR THE DETERMINATION OF THE APPROXIMATE CARDIAC OUTPUT IN MAN

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Although the ethyl iodide method of Starr and Gamble (1) and the acetylene method of Grollman (2) give useful results in the determination of the cardiac output in man, they require special apparatus and a time consuming technique not easy to master. It appeared that if the determination of the cardiac output is to become a practical procedure for the clinic a further simplification of methods is necessary, and with this end in view the possibilities of using oxygen and carbon dioxide for this purpose were reinvestigated. The plan was to devise the simplest possible procedure, to make the necessary assumptions, and then to compare the results with the values secured by means of ethyl iodide and acetylene.

A method has been developed<sup>1</sup> by which the cardiac output may be estimated from a determination of metabolism and the analysis of the oxygen and carbon dioxide contents of only two samples, collected during a single rebreathing procedure much like that used in Grollman's acetylene method. The results obtained by this new oxygen method have been found to be in reasonably good agreement with those secured by the use of ethyl iodide and acetylene.

## METHOD

If assumptions to be discussed hold, the cardiac output can be determined from the solution of two simultaneous equations of the Fick type, certain items of each having been obtained under somewhat different conditions.

For the first equation the normal condition is taken. We have:

$$\text{Cardiac output} = \frac{\text{Normal O}_2 \text{ consumption}}{\text{Normal arterial O}_2 \text{ content} - \text{Normal venous O}_2 \text{ content}} \quad (1, a)$$

For the determination of the corresponding values of the second equation the subject re-

breathes, for a brief period, a mixture low in oxygen; and again we have:

$$\text{Cardiac output} = \frac{\text{O}_2 \text{ consumption during rebreathing}}{\text{Arterial O}_2 \text{ content during rebreathing} - \text{Venous O}_2 \text{ content}} \quad (1, b)$$

On the assumption that the cardiac output and the oxygen content of the venous blood have the same values in the two experiments, the two equations (1, a) and (1, b) may be combined mathematically so as to eliminate the unknown venous oxygen content. The cardiac output is given by the resulting equation:

$$\text{Cardiac output} = \frac{\text{Normal O}_2 \text{ consumption} - \text{O}_2 \text{ consumption during the rebreathing}}{\text{Normal arterial O}_2 \text{ content} - \text{Arterial O}_2 \text{ content during the rebreathing}} \quad (2)$$

## APPARATUS

The apparatus necessary for rebreathing is similar to that employed by Grollman for rebreathing acetylene, except that two three-way valves and two rubber bags are employed, making it almost identical with the apparatus of Gladstone (3).

One needs (1) a mouthpiece connected by (2) a short connecting tube of 3 cm. diameter with side tubes of 4 mm. bore for sampling, leading to (3) the valves, permitting connection of the subject with room air, or with (4) a 4 liter rubber bag, or with (5) a small spirometer or another 4 liter rubber bag. In addition, one needs (6) two Haldane sampling tubes, the upper stopcocks bored to not less than 4 mm.; with mercury, rubber tubing and a reservoir to permit the production of a Toricellian vacuum; (7) a Haldane gas analyzer or one of its modifications, (8) a stop watch, (9) a metronome, (10) rubber connecting tubing, (11) a nose clip and (12) a supply of nitrogen. Finally (13), any standard method for the estimation of metabolic rate may be employed. We use the Benedict-Roth (21), employing the spirometer for the additional purpose mentioned above.

<sup>1</sup> The work was carried out in the Laboratory of Research Therapeutics in the Hospital of the University of Pennsylvania.

It has been found advantageous, although not necessary, to enclose the rebreathing bag in an air-tight metal container having an outlet connected to the Benedict-Roth spirometer, so that a graphic record of changes in the bag volume during filling and rebreathing may be obtained by displacement of air outside the bag.

#### PROCEDURE

Two rebreathing techniques have been employed, the simpler (1), identical with that of Grollman, being used only when the vital capacity is normal. The second (2), much like that of Gladstone (3), is used under other circumstances.

(1) Only one rebreathing bag is employed, filled initially with about 2500 cc. of nitrogen. The metronome is started at from 24 to 30 beats a minute. The subject, at first quietly breathing room air through the mouthpiece, is instructed to make a maximal expiration at command and then to inhale and exhale maximally, keeping time with the metronome. The operator connects the subject with the bag after the first maximal expiration, assures himself that time is being kept and the bag emptied at each inspiration, and collects samples from the last portions of the 6th and 10th expirations, noting the time between collections with the stop watch.

(2) In some patients with low vital capacity the first procedure may yield a lung-bag mixture too high in oxygen. Therefore the following technique was employed.

A preliminary estimation of the approximate vital capacity is made by having the subject inhale various amounts of air from the bag or spirometer after a maximal expiration.

A measured quantity of nitrogen, a little less than the patient could inhale, is placed in bag *A*; a larger, unmeasured amount in bag *B* or in the spirometer.

The rebreathing technique is identical with (1) except that after the first maximal expiration to room air the subject is connected with bag *B*, or the spirometer, for a full respiratory cycle, and then connected with bag *A* for the remainder of the rebreathing. Timed samples are taken from the last portions of the 5th and 9th expirations into bag *A*. The samples are analyzed for oxygen and carbon dioxide in the usual manner.

The metabolic rate is estimated either before or after the rebreathing.

#### CALCULATION OF RESULTS

This can be illustrated best by employing data obtained from a typical experiment. All volumes given are for dry gas under standard conditions. We have:

1. Normal oxygen consumption = 260 cc. per minute.

2. Normal arterial oxygen content. The oxygen capacity of the blood may be assumed to be 200 cc. per liter if the subject is normal, or may be estimated from the hemoglobin concentration. The arterial blood is as-

sumed to be 96 per cent saturated. In this instance normal arterial oxygen content is 192 cc. per liter.

#### 3. Oxygen consumption during the rebreathing.

Nitrogen in rebreathing bag initially = 2310 cc.

Assumed residual air = 1125 cc.<sup>2</sup>

Total initial lung-bag volume = 3435 cc.

The average lung-bag volume during the rebreathing is assumed equal to the initial lung-bag volume.

Time between collection of samples = 9.8 seconds.

By analysis	CO <sub>2</sub> per cent	O <sub>2</sub> per cent	N <sub>2</sub> per cent
First sample . . . . .	4.98	5.14	89.88
Second sample . . . . .	5.57	5.04	89.39
Average percentage . . . . .	5.26	5.09	
Average tension (saturated at 37° C., barometer 766) . . . .	37.9 mm.	36.5 mm.	

$$\begin{aligned}
 &\text{Oxygen consumption during the rebreathing} \\
 &= (\text{lung-bag volume}) \times (\text{change of oxygen percentage in the lung-bag system}) \\
 &\quad \times \left( \frac{60}{\text{seconds between collection of samples}} \right) \\
 &= (3435)(0.10) \left( \frac{60}{9.8} \right) \\
 &= 21 \text{ cc. per minute, dry under standard conditions.}
 \end{aligned}$$

4. Arterial oxygen content during the rebreathing. The nomogram of Henderson, et al. (5) is consulted. The percentage oxygen saturation is determined from the average oxygen and carbon dioxide tensions of the two samples. In this instance it is 72.5 per cent. Hence:

Arterial oxygen content = 72.5 × 200 = 145 cc. per liter.

5. Calculation of cardiac output. Substituting the preceding four values in Equation 2:

$$\text{Cardiac output} = \frac{260 - 21}{192 - 145} = 5.1 \text{ liters per minute.}$$

#### MAGNITUDE OF ERRORS INHERENT IN ASSUMPTIONS AND TECHNIQUE

*Influence of increases in the cardiac output during the rebreathing, and of errors in the assumed value of residual air*

In combining the expressions evaluated by the two determinations of oxygen consumption as two simultaneous equations (Equations 1a, b and 2 under "method"), the equality of the cardiac outputs under the two conditions has been assumed.

This assumption introduces an error into the results because the acceleration of the circulation known to result from rebreathing will give an oxygen consumption which is erroneously high.

<sup>2</sup> From the work of Hurtado et al. (4), their average value being 1360 cc., saturated at 37° C.

Fortunately, however, the low oxygen tension during the rebreathing makes the corresponding oxygen consumption, the second term in the numerator of Equation 2, very small. Large percentage errors in this term, therefore, can have but little effect upon the calculated cardiac output. For the same reason, an error in the volume of the residual air, assumed for the calculation of this oxygen consumption during rebreathing, will produce a negligible effect upon the results.

That these errors are small may perhaps be understood more easily from a consideration of Figure 1. For a representative subject the oxy-

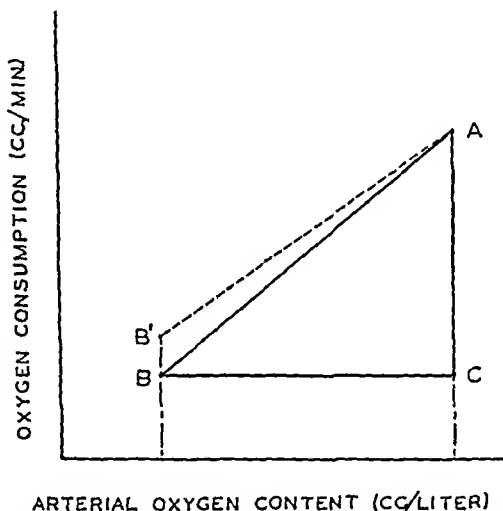


FIG. 1. THE ERROR RESULTING FROM AN ALTERATION IN CARDIAC OUTPUT DURING THE REBREATHING

The cardiac output may be represented by the slope of the line  $AB$  (see text). An increase of the cardiac output during the rebreathing decreases the calculated result to the value of the slope of the line  $AB'$ . The error is small, however, if the oxygen tension in the lungs during the rebreathing is not far from the oxygen tension of the mixed venous blood, a condition which may conveniently be attained experimentally.

gen consumption of Equation 1a, is plotted at point  $A$  as a function of the assumed arterial oxygen content of 192 cc. per liter. The quantities of Equation 1b, i.e., the oxygen consumption measured during a rebreathing experiment and its corresponding arterial oxygen content, are plotted as point  $B$ . If the line  $BC$  is drawn parallel to the axis of abscissae it will be seen that the line  $(A-C)$  represents the numerator of the right hand side of Equation (2) and the line  $(C-B)$  its denominator. Therefore, from Equation 2,

$(A-C)/(C-B)$ , or the slope of the line  $AB$ , represents the cardiac output.

It is obvious that this error in the cardiac output, for any given percentage error  $(B'-B)$  in the oxygen consumption during the rebreathing, becomes less as the rebreathing is performed at an oxygen tension nearer equilibrium with the mixed venous blood, for as the absolute value of the ordinate at  $B$  (the oxygen consumption) decreases, the difference  $(B'-B)$  must decrease and the slope of the line  $AB'$  (measured cardiac output) approaches the slope of  $AB$  (true cardiac output).

Since the mixture rebreathed consists initially of nitrogen alone, the oxygen tension in the lungs is never such as to make the oxygen consumption during the rebreathing experiment more than a small fraction of the consumption measured at the normal alveolar oxygen levels. Thus, with the normal subjects studied by the first rebreathing technique (noted above under "Procedure") the average oxygen consumption during the experiments was 9.5 per cent of the average basal oxygen consumption of these subjects. Similarly, for the clinical subjects studied by the second rebreathing technique the corresponding value was 14 per cent.

To study the errors resulting, in actual practice, from the increase of cardiac output during the rebreathing, additional experiments were performed upon the first 9 of the normal subjects of Table I, in each case on the same day and after an additional 20 minute rest period. After a maximal expiration to the room the subject rebreathed a measured volume of a mixture of about 50 per cent oxygen in nitrogen. The residual air volumes were then determined from samples of the last portions of the initial maximal expirations to the room and of the first inspirations from the bag. Knowing the residual airs, timed samples of the 6th, and 10th or 11th expirations to the bag permitted the determination of the oxygen consumptions during the rebreathing. The results are compared with the basal oxygen consumptions in Table II, and the average increase of oxygen consumption during the rebreathing, which has been assumed to be the average increase of corresponding cardiac output, was found to be 66 per cent. Recomputation of the values of cardiac output for the 9 subjects, correcting the

It has been found advantageous, although not necessary, to enclose the rebreathing bag in an air-tight metal container having an outlet connected to the Benedict-Roth spirometer, so that a graphic record of changes in the bag volume during filling and rebreathing may be obtained by displacement of air outside the bag.

#### PROCEDURE

Two rebreathing techniques have been employed, the simpler (1), identical with that of Grollman, being used only when the vital capacity is normal. The second (2), much like that of Gladstone (3), is used under other circumstances.

(1) Only one rebreathing bag is employed, filled initially with about 2500 cc. of nitrogen. The metronome is started at from 24 to 30 beats a minute. The subject, at first quietly breathing room air through the mouthpiece, is instructed to make a maximal expiration at command and then to inhale and exhale maximally, keeping time with the metronome. The operator connects the subject with the bag after the first maximal expiration, assures himself that time is being kept and the bag emptied at each inspiration, and collects samples from the last portions of the 6th and 10th expirations, noting the time between collections with the stop watch.

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A preliminary estimation of the approximate vital capacity is made by having the subject inhale various amounts of air from the bag or spirometer after a maximal expiration.

A measured quantity of nitrogen, a little less than the patient could inhale, is placed in bag *A*; a larger, unmeasured amount in bag *B* or in the spirometer.

The rebreathing technique is identical with (1) except that after the first maximal expiration to room air the subject is connected with bag *B*, or the spirometer, for a full respiratory cycle, and then connected with bag *A* for the remainder of the rebreathing. Timed samples are taken from the last portions of the 5th and 9th expirations into bag *A*. The samples are analyzed for oxygen and carbon dioxide in the usual manner.

The metabolic rate is estimated either before or after the rebreathing.

#### CALCULATION OF RESULTS

This can be illustrated best by employing data obtained from a typical experiment. All volumes given are for dry gas under standard conditions. We have:

1. Normal oxygen consumption = 260 cc. per minute.

2. Normal arterial oxygen content. The oxygen capacity of the blood may be assumed to be 200 cc. per liter if the subject is normal, or may be estimated from the hemoglobin concentration. The arterial blood is as-

sumed to be 96 per cent saturated. In this instance normal arterial oxygen content is 192 cc. per liter.

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Nitrogen in rebreathing bag initially = 2310 cc.

Assumed residual air = 1125 cc.<sup>2</sup>

Total initial lung-bag volume = 3435 cc.

The average lung-bag volume during the rebreathing is assumed equal to the initial lung-bag volume.

Time between collection of samples = 9.8 seconds.

By analysis	CO <sub>2</sub> per cent	O <sub>2</sub> per cent	N <sub>2</sub> per cent
First sample . . . . .	4.98	5.14	89.88
Second sample . . . . .	5.57	5.04	89.39
Average percentage . . . . .	5.26	5.09	
Average tension (saturated at 37° C., barometer 766) . . . . .	37.9 mm.	36.5 mm.	

$$\begin{aligned} \text{Oxygen consumption during the rebreathing} &= (\text{lung-bag volume}) \times (\text{change of oxygen percentage in the lung-bag system}) \\ &\times \left( \frac{60}{\text{seconds between collection of samples}} \right) \\ &= (3435)(0.10) \left( \frac{60}{9.8} \right) \\ &= 21 \text{ cc. per minute, dry under standard conditions.} \end{aligned}$$

4. Arterial oxygen content during the rebreathing. The nomogram of Henderson, et al. (5) is consulted. The percentage oxygen saturation is determined from the average oxygen and carbon dioxide tensions of the two samples. In this instance it is 72.5 per cent. Hence:

Arterial oxygen content =  $72.5 \times 200 = 145$  cc. per liter.

5. Calculation of cardiac output. Substituting the preceding four values in Equation 2:

$$\text{Cardiac output} = \frac{260 - 21}{192 - 145} = 5.1 \text{ liters per minute.}$$

#### MAGNITUDE OF ERRORS INHERENT IN ASSUMPTIONS AND TECHNIQUE

*Influence of increases in the cardiac output during the rebreathing, and of errors in the assumed value of residual air*

In combining the expressions evaluated by the two determinations of oxygen consumption as two simultaneous equations (Equations 1a, b and 2 under "method"), the equality of the cardiac outputs under the two conditions has been assumed.

This assumption introduces an error into the results because the acceleration of the circulation known to result from rebreathing will give an oxygen consumption which is erroneously high.

<sup>2</sup> From the work of Hurtado et al. (4), their average value being 1360 cc., saturated at 37° C.

workers, Grollman and Marshall (7) in particular. Our data confirm these findings.

Samples of successive inspirations and expirations were taken in control experiments in which the normal subjects rebreathed initial mixtures of 2000 to 3000 cc. of nitrogen. Plotting the nitrogen percentages in the samples as a function of the number of respiratory cycles, it was found, as would be expected, that after the 3d or 4th cycle the points representing inspirations and those representing expirations arranged themselves on parallel and slightly converging lines as in Figure 2, a result similar to that of Lundsgaard and

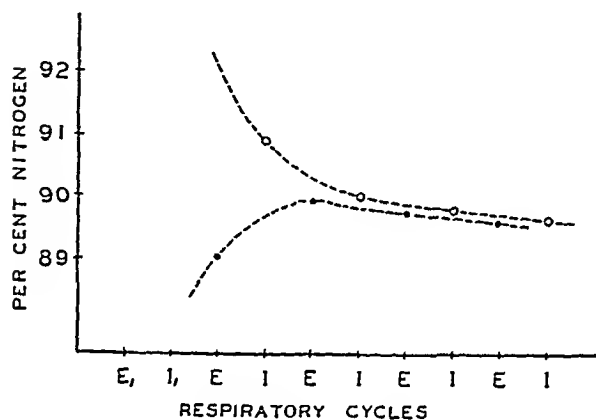


FIG. 2. NITROGEN PERCENTAGES DURING THE PERIOD OF MIXING

This figure is a representation of several experiments in which normal subjects rebreathed initial mixtures of pure nitrogen. The dots indicate the nitrogen percentages in successive expirations, beginning with  $E_1$ , the initial maximal expiration to the room. The circles are the nitrogen percentages found in samples collected from the last portions of successive inspirations, the initial inspiration,  $I_1$ , being 100 per cent.

Schierbeck (8). (The average nitrogen content was decreasing due to the effect of the blowing off of carbon dioxide.) The nitrogen percentages in the bag samples were slightly above those of the corresponding preceding expirations, since each bag sample was an average of the preceding expiration. This circumstance, found to hold when mixing could be assumed from the results of other workers, was taken as a rough criterion of mixing in the lung-bag system. Additional samples, collected during determinations of cardiac output on 5 out of the 7 clinical subjects studied, satisfied this criterion and demonstrated that there was no

serious inadequacy of mixing at the time of collection of the first sample used for the calculation of the cardiac output.

#### *Recirculation and the permissible duration of re-breathing*

Discussion of recirculation will be limited to a consideration of significant changes in the oxygen content of the blood returning to the lungs during the course of the rebreathing experiments. In procedures of the nature used here, Donal and Gamble (9) found from the inflections of their carbon dioxide-oxygen tension curves an average recirculation time of 24.5 seconds with ten normal subjects lying at rest. In this investigation a slightly less sensitive but more practical test was employed, in that the values of cardiac output were computed from successive pairs of samples collected during the rebreathing experiments, and significant recirculation was considered to have occurred when the calculated cardiac output decreased more than 10 per cent, as determined from the later sample collections. This does not, of course, distinguish between recirculation and a possible further acceleration in the blood flow during the rebreathing. However, such a distinction is not necessary for present purposes, since a change in the calculated cardiac output from either cause is equally undesirable.

For the 11 normal subjects studied in this investigation the average time of collection of the last sample used for the determination of cardiac output was 23 seconds. With 6 of these subjects a 3d sample was collected from the expiration made two cycles later. Since with only one subject did a significant change (—15 per cent) in the calculated cardiac output result from a continuation of the rebreathing for two extra cycles, it is believed that the technique prescribed for normal subjects will not, in general, result in errors due to recirculation.

For the 12 experiments with the 7 clinical subjects studied, the average time of collection of the last sample used for the calculation of cardiac output, taken from the 9th expiration to the bag, was 28 seconds. In 7 of the experiments, with 6 subjects, additional samples showed that in only one case could there have been significant recirculation between the 7th and 9th cycles, the cardiac output change being —17 per cent. It is believed, there-

oxygen consumptions for the average increase of 66 per cent during the rebreathings, has shown that the results by our oxygen method must have been about 6 per cent too low.

TABLE I  
*Values of cardiac output*

Subject	Ethyl iodide method	Acetylene method	Oxygen method
	<i>liters per minute</i>	<i>liters per minute</i>	<i>liters per minute</i>
NORMAL SUBJECTS			
1	2.6, 3.0	3.7	3.8
2	4.3, 3.3	4.0	3.6
3	5.2, 4.6	4.7	3.2
4	3.8, 4.2	5.7	3.5
5	3.6, 3.5	3.6	3.2
6	6.9, 7.9	7.1	5.3
7	4.1, 3.8	3.8	4.4
8	4.8, 4.9	4.0	5.9
9	3.2, 2.6	3.2	2.6
10	4.0, 4.2	3.6	4.2, 4.1
11	4.4	4.0	4.5
Averages:	4.23	4.30	4.00

CLINICAL SUBJECTS

Subject	Ethyl iodide method	Oxygen method	Hemoglobin	Basal oxygen consumption
	<i>liters per minute</i>	<i>liters per minute</i>	<i>per cent</i>	<i>cc. per minute</i>
12	2.6, 3.0	2.8, 3.0	76	169
13	6.0, 5.9	6.2, 6.6	52	279
14	5.3, 6.3	7.4	46	257
15	3.5, 2.8	3.0, 2.7	100	169
16	5.1	5.3, 4.8	100	241
17	3.0, 4.1	3.0, 2.6	95	176
18	4.4	3.9	100	266
Averages:	4.39	4.47		

TABLE II  
*Additional data on nine of the subjects of Table I*

Subject	Oxygen consumptions			Measured residual air (dry under standard conditions)	Cardiac output by holding-breath procedure
	Basal	Rebreathing high oxygen	Increase		
	<i>cc. per minute</i>	<i>cc. per minute</i>	<i>per cent</i>	<i>cc.</i>	<i>liters per minute</i>
1	246	412	67	1316	3.8
2	239	466	95	1248	3.7
3	257	426	66	1450	4.3
4	249	515	107	1720	4.0
5	217	380	75	1000	3.3
6	279	471	69	1640	5.0
7	251	351	40	1180	4.3
8	268	404	50	1235	5.9
9	196	246	26	1130	3.2
Averages:	245	408	66	1324	4.17

It will be seen from Table II that the measured values of residual air of the first 9 normal subjects averaged 18 per cent higher than the assumed value of 1125 cc. used in computing the results of Table I. However, when the results for these subjects were recalculated, using the measured residual airs, the average reduction in the values of cardiac output was less than 1 per cent.

In order to determine the effect of a reduction in the respiratory gymnastics incident to the method, additional experiments at low oxygen tensions were performed upon the above 9 basal subjects. The procedure of holding the breath according to Krogh and Lindhard (6) was employed, together with a modification of the mixing technique of Gladstone (3). The subject expired maximally to air at zero time, emptied a bag containing about 3600 cc. of nitrogen, discarded about 1200 cc. to the room, completed the maximal expiration to the bag and continued to rebreathe. The third expiration to the bag was limited to about 1200 cc., the breath was held in this position of partial expiration for 8 to 10 seconds, and the maximal expiration to the bag was completed. Timed samples were collected from the last portions of the partial expirations before and after holding the breath. The results are shown in Column 6 of Table II and may be compared with those for the same subjects in Column 4 of Table I, since the corresponding experiments were performed on the same day with only 30 minutes to an hour intervening. The average results were not significantly different, as may be seen by inspection of the Tables. The slight average increase of about 4 per cent was in the expected direction for, due to the deeper initial inspiration of nitrogen, the average oxygen consumption in the experiments in which the breath was held was less than 1 per cent of the average basal rate, compared with 9.5 per cent for the rebreathings yielding the results of Table I.

#### *Mixing in the lung-bag system*

No extensive investigation was made of the adequacy of mixing at the time of collection of the first cardiac output sample, since under similar respiratory conditions mixing in the lung-bag system has been found to be complete by other

put was again determined by the acetylene method of Grollman (2) in all normal subjects.<sup>4</sup>

After an additional period of 30 minutes rest the oxygen rebreathing procedure was carried out. Still later, supplementary experiments which are described in the preceding section were performed on many of the subjects for the purpose of investigating errors.

In Table I, the results secured by the method described have been compared with those obtained by the ethyl iodide and acetylene procedures. It is concluded from a statistical analysis that there are no significant differences between the averages of the results obtained by means of the three methods.

#### DISCUSSION

Reviews of the oxygen and carbon dioxide methods, founded on the principle of Fick, have been given by Grollman (12) and by Richards and Strauss (14). Equilibration of the venous blood with the tensions of oxygen, carbon dioxide, or both gases in the lung-bag system, in the methods such as those of Barcroft, Roughton and Shoji (15), Douglas and Haldane (16) or Burwell and Robinson (17), was a tedious procedure and the difficulties of such an equilibration have been pointed out by Richards and Strauss (14). The equilibration of *oxygenated* venous blood with the carbon dioxide tension of the gas in the lung-bag system offers obvious advantages, among them that the rate of carbon dioxide transport between the blood and gas is proportional to the difference in the corresponding tensions. Advantage of this fact was taken by Fridericia (18) and by Liljestrand and Lindhard (19).

The "triple extrapolation" method of Redfield, Bock and Meakins (20) represented a simplification of technique in that the tensions of the gases in the mixed venous blood were inferred, by extrapolation, from analyses of mixtures having quite different gas tensions. It has been shown by Donal and Gamble (9), however, that this procedure may lead to erroneous results.

The method here described is much simpler than any of those mentioned above. Various elaborations of it are possible, for instance the

normal arterial oxygen tension might be determined by analysis of blood obtained by arterial puncture. Theoretically, carbon dioxide might be used instead of oxygen, but the many physiological factors influencing the behavior of carbon dioxide in the body make the use of oxygen much to be preferred.

It might be considered desirable, when working with clinical subjects of low vital capacity, to employ two initial breaths of nitrogen before connecting the subject to the final rebreathing bag, in order to reduce the average oxygen tension in the lungs and hence the oxygen consumption during the period between the collection of the timed samples. Or, in order to shorten the time necessary for mixing, other and more complicated rebreathing procedures might be employed. In both cases, however, preliminary experiments have shown that the purely mechanical difficulties of application of such techniques render their value questionable, although they may offer theoretical advantages.

Some question might be raised about the propriety of asking abnormal subjects to inhale nitrogen for about 25 seconds. As is well known there are no subjective sensations from this procedure. Cyanosis has not been observed in our normal subjects. It was seen in one patient at the extreme end of the rebreathing period. Untoward possibilities in this direction should be kept in mind by those planning to use the method in cardiac or pulmonary disease.

Obviously, further experience with the method might indicate the desirability of the use of other values for the average residual air, or other blood nomograms, than those employed in this investigation.

The agreement between the average results obtained by this oxygen method and those secured by the ethyl iodide and acetylene procedures is far from perfect but it is satisfactory enough. In certain individuals the agreement between the results by the three methods is good, in others it is poor. This is in accord with expectations for, when duplicate analyses are made by a single method, some subjects give constant results while others are far more variable. The application of the three different techniques required far more time than that which must separate duplicate estimations. Most subjects cannot be expected to

<sup>4</sup> Dr. Clarence James Gamble has kindly permitted the presentation of these as yet unpublished acetylene results which formed a portion of a separate investigation.



fore, that the technique prescribed for resting clinical subjects will not extend into the period of recirculation, particularly since the average time required for the procedure could probably be substantially reduced with more experience on the part of the operator.

#### *Miscellaneous assumptions and possibilities of error*

The determination of the oxygen dissociation curves of the blood of each subject is believed to be unnecessary and in this investigation the nomogram of Henderson, et al. (5) has been employed throughout. Comparative calculations made with other nomograms have shown that *changes* in the cardiac output of a subject may be determined with sufficient accuracy by the use of any standard nomogram. The absolute values of the cardiac output of 9 subjects were systematically decreased by an average of 10 per cent when recomputed from the nomogram of Dill, et al. (10, page 207).

From Equation 2 the values of cardiac output vary inversely as the oxygen capacities of the blood. For normal subjects the use of an assumed blood oxygen capacity of 200 cc. per liter has been found, with 9 subjects, to introduce an average error of only  $\pm 3$  per cent into the results. For clinical subjects the oxygen capacities have been determined from hemoglobin estimations.

Since it is impracticable to determine the oxygen saturation of the arterial blood of each subject, the value of 96 per cent has been assumed from the work of Bock et al. (11). A variation of 1 per cent in this assumption will introduce an average error of between 3 and 4 per cent into the calculated cardiac output.

The two determinations of oxygen consumption are performed under identical conditions and are separated by only a short period of time. The work of Grollman (12) and others encourages the belief that this will result in identity of venous oxygen contents. That the venous oxygen content 15 to 25 seconds after beginning to rebreathe is the same as that immediately before the rebreathing begins is supported by the findings of Donal and Gamble (9), and of this investigation, that only occasionally does blood low in oxygen content return to the lungs earlier than about 24 seconds after the start of the rebreathing procedure.

It is believed unnecessary to take account of the absorption of nitrogen from the lungs during the low oxygen rebreathing experiment, since the average increase, although systematic, in the calculated cardiac output resulting from the neglect of this factor can be shown to be less than 1 per cent. If account is taken of the changes in the lung-bag volume during the low oxygen rebreathing experiment, by making use of the altered nitrogen percentage in the second sample as compared with the first, the cardiac outputs of 9 normal subjects have been found to be increased an average of 3 per cent. Although this effect is also systematic, since more carbon dioxide is ordinarily excreted during the period between the sample collections than there is oxygen absorbed, the error is small and is somewhat reduced by the absorption of nitrogen mentioned above. Therefore, for simplicity, this volume change has been neglected in this investigation.<sup>3</sup>

Calculations have shown that no appreciable error is introduced by assuming, in Equation 2, that the entire oxygen consumption during the rebreathing takes place at the average gas tensions found in the samples.

#### EXPERIMENTAL RESULTS

The values of cardiac output obtained by the simplified oxygen method were compared with the results secured on the same subjects by means of ethyl iodide and acetylene.

Two determinations of the basal cardiac output of each subject were made, about 20 minutes apart, by the ethyl iodide method of Starr and Gamble (1), using the katharometer as described by Donal, Gamble and Shaw (13). During each determination, duplicate estimations were made of oxygen consumption, of carbon dioxide elimination, and of the oxygen and carbon dioxide tensions of alveolar air.

About 20 minutes later the basal cardiac out-

<sup>3</sup> Correction for this change may be made by merely multiplying the oxygen percentage in the second sample by the ratio of the nitrogen percentage in the first sample to that of the second sample. Thus:

$$\text{Corrected } (O_2)_{II} = \text{Measured } (O_2)_{II} \times \frac{(N_2)_I}{(N_2)_{II}},$$

where the Roman subscripts refer to the respective samples.



put was again determined by the acetylene method of Grollman (2) in all normal subjects.<sup>4</sup>

After an additional period of 30 minutes rest the oxygen rebreathing procedure was carried out. Still later, supplementary experiments which are described in the preceding section were performed on many of the subjects for the purpose of investigating errors.

In Table I, the results secured by the method described have been compared with those obtained by the ethyl iodide and acetylene procedures. It is concluded from a statistical analysis that there are no significant differences between the averages of the results obtained by means of the three methods.

#### DISCUSSION

Reviews of the oxygen and carbon dioxide methods, founded on the principle of Fick, have been given by Grollman (12) and by Richards and Strauss (14). Equilibration of the venous blood with the tensions of oxygen, carbon dioxide, or both gases in the lung-bag system, in the methods such as those of Barcroft, Roughton and Shoji (15), Douglas and Haldane (16) or Burwell and Robinson (17), was a tedious procedure and the difficulties of such an equilibration have been pointed out by Richards and Strauss (14). The equilibration of *oxygenated* venous blood with the carbon dioxide tension of the gas in the lung-bag system offers obvious advantages, among them that the rate of carbon dioxide transport between the blood and gas is proportional to the difference in the corresponding tensions. Advantage of this fact was taken by Fridericia (18) and by Liljestrand and Lindhard (19).

The "triple extrapolation" method of Redfield, Bock and Meakins (20) represented a simplification of technique in that the tensions of the gases in the mixed venous blood were inferred, by extrapolation, from analyses of mixtures having quite different gas tensions. It has been shown by Donal and Gamble (9), however, that this procedure may lead to erroneous results.

The method here described is much simpler than any of those mentioned above. Various elaborations of it are possible, for instance the

normal arterial oxygen tension might be determined by analysis of blood obtained by arterial puncture. Theoretically, carbon dioxide might be used instead of oxygen, but the many physiological factors influencing the behavior of carbon dioxide in the body make the use of oxygen much to be preferred.

It might be considered desirable, when working with clinical subjects of low vital capacity, to employ two initial breaths of nitrogen before connecting the subject to the final rebreathing bag, in order to reduce the average oxygen tension in the lungs and hence the oxygen consumption during the period between the collection of the timed samples. Or, in order to shorten the time necessary for mixing, other and more complicated rebreathing procedures might be employed. In both cases, however, preliminary experiments have shown that the purely mechanical difficulties of application of such techniques render their value questionable, although they may offer theoretical advantages.

Some question might be raised about the propriety of asking abnormal subjects to inhale nitrogen for about 25 seconds. As is well known there are no subjective sensations from this procedure. Cyanosis has not been observed in our normal subjects. It was seen in one patient at the extreme end of the rebreathing period. Untoward possibilities in this direction should be kept in mind by those planning to use the method in cardiac or pulmonary disease.

Obviously, further experience with the method might indicate the desirability of the use of other values for the average residual air, or other blood nomograms, than those employed in this investigation.

The agreement between the average results obtained by this oxygen method and those secured by the ethyl iodide and acetylene procedures is far from perfect but it is satisfactory enough. In certain individuals the agreement between the results by the three methods is good, in others it is poor. This is in accord with expectations for, when duplicate analyses are made by a single method, some subjects give constant results while others are far more variable. The application of the three different techniques required far more time than that which must separate duplicate estimations. Most subjects cannot be expected to

<sup>4</sup> Dr. Clarence James Gamble has kindly permitted the presentation of these as yet unpublished acetylene results which formed a portion of a separate investigation.

fore, that the technique prescribed for resting clinical subjects will not extend into the period of recirculation, particularly since the average time required for the procedure could probably be substantially reduced with more experience on the part of the operator.

#### *Miscellaneous assumptions and possibilities of error*

The determination of the oxygen dissociation curves of the blood of each subject is believed to be unnecessary and in this investigation the nomogram of Henderson, et al. (5) has been employed throughout. Comparative calculations made with other nomograms have shown that *changes* in the cardiac output of a subject may be determined with sufficient accuracy by the use of any standard nomogram. The absolute values of the cardiac output of 9 subjects were systematically decreased by an average of 10 per cent when recomputed from the nomogram of Dill, et al. (10, page 207).

From Equation 2 the values of cardiac output vary inversely as the oxygen capacities of the blood. For normal subjects the use of an assumed blood oxygen capacity of 200 cc. per liter has been found, with 9 subjects, to introduce an average error of only  $\pm 3$  per cent into the results. For clinical subjects the oxygen capacities have been determined from hemoglobin estimations.

Since it is impracticable to determine the oxygen saturation of the arterial blood of each subject, the value of 96 per cent has been assumed from the work of Bock et al. (11). A variation of 1 per cent in this assumption will introduce an average error of between 3 and 4 per cent into the calculated cardiac output.

The two determinations of oxygen consumption are performed under identical conditions and are separated by only a short period of time. The work of Grollman (12) and others encourages the belief that this will result in identity of venous oxygen contents. That the venous oxygen content 15 to 25 seconds after beginning to rebreathe is the same as that immediately before the rebreathing begins is supported by the findings of Donal and Gamble (9), and of this investigation, that only occasionally does blood low in oxygen content return to the lungs earlier than about 24 seconds after the start of the rebreathing procedure.

It is believed unnecessary to take account of the absorption of nitrogen from the lungs during the low oxygen rebreathing experiment, since the average increase, although systematic, in the calculated cardiac output resulting from the neglect of this factor can be shown to be less than 1 per cent. If account is taken of the changes in the lung-bag volume during the low oxygen rebreathing experiment, by making use of the altered nitrogen percentage in the second sample as compared with the first, the cardiac outputs of 9 normal subjects have been found to be increased an average of 3 per cent. Although this effect is also systematic, since more carbon dioxide is ordinarily excreted during the period between the sample collections than there is oxygen absorbed, the error is small and is somewhat reduced by the absorption of nitrogen mentioned above. Therefore, for simplicity, this volume change has been neglected in this investigation.<sup>3</sup>

Calculations have shown that no appreciable error is introduced by assuming, in Equation 2, that the entire oxygen consumption during the rebreathing takes place at the average gas tensions found in the samples.

#### EXPERIMENTAL RESULTS

The values of cardiac output obtained by the simplified oxygen method were compared with the results secured on the same subjects by means of ethyl iodide and acetylene.

Two determinations of the basal cardiac output of each subject were made, about 20 minutes apart, by the ethyl iodide method of Starr and Gamble (1), using the katharometer as described by Donal, Gamble and Shaw (13). During each determination, duplicate estimations were made of oxygen consumption, of carbon dioxide elimination, and of the oxygen and carbon dioxide tensions of alveolar air.

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maintain a completely constant basal cardiac output over a period of about an hour and a half while undergoing different experiments of an unfamiliar type.

The more elaborate methods of other workers have been evolved to avoid some of the assumptions accepted in our simple procedure. But, in order to avoid these, other assumptions have been accepted and the question must be asked whether anything substantial has been gained by the more elaborate techniques.

#### SUMMARY

A simplified oxygen method has been developed by which the cardiac output of either normal or clinical subjects may be estimated from a determination of metabolism and the analysis of the oxygen and carbon dioxide contents of only two samples, collected during a single rebreathing procedure.

The effects of various errors inherent in the assumptions and technique have been investigated. Experimental results have shown that many of these errors are of such small magnitude that they may be neglected. From calculations, and from a consideration of the work of other investigators, it has been concluded that the influence of the remaining apparent errors is likewise relatively unimportant.

The averages of estimations of basal cardiac output by the new procedure have been found to be in good agreement with the averages of determinations made on the same normal and clinical subjects by the ethyl iodide method of Starr and Gamble and by the acetylene method of Grollman. The agreement of estimations by the three methods on individual subjects is not very good, a result to be expected from the known variation of duplicate estimations in many subjects. In seven instances the result of the oxygen method agreed more closely with the average of the ethyl iodide results than with the result of the single estimation by acetylene. In three instances the oxygen result was closer to that obtained by acetylene.

Duplicate estimations by the oxygen method agreed more closely than similar duplicates made by ethyl iodide in four patients.

I make grateful acknowledgment to Dr. Clarence James Gamble and Professor Isaac Starr for their constant encouragement and fruitful suggestions during the course of this investigation, and for their valuable help in the preparation of this manuscript for publication.

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## CHRONIC PYELONEPHRITIS AND ARTERIAL HYPERTENSION

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The present paper presents certain clinical and pathological evidence which demonstrates that hypertension not infrequently is associated with pyelonephritis before there is any appreciable diminution in renal function and that hypertension which is secondary to unilateral pyelonephritis may disappear when the involved kidney is removed.

Ritter and Baehr (1) described renal arteriolar sclerosis in congenital polycystic disease of the kidney and remarked upon a preliminary period of arterial hypertension, cardiac hypertrophy and hyposthenuria that usually precedes the terminal uremia in that disease. Bell and Pedersen (2) stated that "hypertension has never been reported in pyelonephritis." Volhard (3) and Schwarz (4) reported hypertension in patients with contracted kidneys (*schrumpfnieren*). Longcope and Winkenwerder (5) reported elevated blood pressures in the uremic phase of cases of chronic pyelonephritis. Weiss, Parker and Robb (6) observed that patients with malignant hypertension frequently had a history of chronic pyelonephritis, pyelitis, or perinephritic abscess. They suggested that such a renal infection may heal but that the hypertension initiated by it may continue to progress. Fishberg (7) mentioned the hypertension that may occur in children in the presence of urinary obstruction and in polycystic disease of the kidney when there is extensive destruction of renal parenchyma. He stated, however, that hypertension does not occur in that disease if there are extensive areas of intact parenchyma. Peters (8), Peters, Laviates and Zimmerman (9), and Zimmerman and Peters (10) have called attention to the frequency with which pyuria and eclampsia are associated in pregnancy and suggested a relation between urinary tract infection and hypertension. Kimmelstiel and Wilson (11) studied thirteen patients with acute diffuse pyelonephritis; nine died in uremia, and hypertension was present in four of these. Two patients presented what

was interpreted as essential hypertension with superimposed diffuse acute pyelonephritis but without renal insufficiency. Twenty-six patients who suffered from diffuse chronic pyelonephritis were also studied; of these, hypertension and uremia were associated in sixteen; hypertension alone was present in four, and uremia without hypertension in six. Hypertension without marked renal insufficiency, therefore, was present in six of their patients. In the majority of instances, they were unable to decide whether they were dealing with a primary "vascular" hypertension or with a secondary "renal" hypertension.

In spite of the frequency with which pyelonephritis is encountered in childhood (12), we have found no report of a serious hypertension occurring in the pyelonephritis of childhood before renal insufficiency was present. Interestingly enough, Amberg (13) in reporting twenty-five cases of hypertension in children included five patients with pyuria or bacilluria but made no particular comment upon the presence of pyelonephritis in these patients.

The records concerning the blood pressures of many of the patients admitted to this hospital with chronic pyelonephritis are not complete enough to supply accurate information concerning the respective times at which the hypertension, if present, and renal insufficiency first appeared, but such data as are available seem significant. During the past ten years fifteen children between three and eleven years of age were shown at necropsy to have pyelonephritis. Adequate records of the blood pressures of seven of these patients are not available. The records of the blood pressures for the remaining eight patients show systolic pressures ranging from 250 to 140 mm. Hg and diastolic pressures from 170 to 110 mm. Hg, the average systolic and diastolic pressures being respectively, 190 and 140 mm. Hg. Two of these patients (Cases 3 and 4 reported below) had hypertensive crises and died of cardiac failure before

significant nitrogen retention occurred. The clinical histories of two others of the group studied pathologically indicated that the pyelonephritis and hypertension preceded severe nitrogen retention. During the same ten year period three patients with pyelonephritis and hypertension died and permission for autopsy was not obtained. The histories of two of these patients (Cases 1 and 2 below) indicate that the pyelonephritis and hypertension preceded significant renal insufficiency and nitrogen retention. During this same

definite proof that the pyelonephritis preceded the hypertension.

The fifth case reported here is that of a patient who, coincident with a ureteral calculus, was found to have a unilateral pyelonephritis and during the course of the next 8 months developed hypertension and cardiac failure. The removal of the one infected kidney was followed by clearing of the urine and a return of the blood pressure to normal where it has remained for 20 months. In this case there is strong evidence that the pye-



FIG. 1. PHOTOMICROGRAPH OF MICROSCOPIC SECTION PREPARED FROM KIDNEY OF CASE 4

Hematoxylin and eosin. Reduced from a magnification of 240 diameters. Note prominence and thickening of walls of small arterioles, interstitial infiltration and casts in the renal tubules. Both chronic pyelonephritis and nephrosclerosis were present in the various sections examined.

period nine patients with pyelonephritis and hypertension were admitted to the hospital and when last seen were living. Of these patients only one had renal insufficiency, and in this one the pyelonephritis and hypertension preceded the appearance of the diminished renal function.

Thus we have fifteen patients (six dead and nine living) who have had chronic pyelonephritis and hypertension over a period of years before there was appreciable diminution in kidney function. The detailed records of four of these patients are given below. In these cases there is no

pyelonephritis preceded the hypertension and in some way had a causal relation to it.

Subsequently, another patient, Case 6, who had a unilateral pyelonephritis and hypertension which was relieved by right nephrectomy, and whose history suggested a relation between the renal lesion and hypertension, was seen on the pediatric ward of the Massachusetts General Hospital through the kindness of Dr. Harold Higgins and Dr. J. D. Barney.<sup>1</sup>

<sup>1</sup>A full report of this patient will be made by Dr. Barney. It is through his kindness that a brief description is given with the cases reported here.



## DISCUSSION

It is of particular interest in relation to the last two patients that Moritz (14) reported three patients with essential hypertension in each of whom the renal arteriolar sclerosis was found at necropsy to be limited to one kidney.

Both chronic pyelonephritis and nephrosclerosis were revealed by postmortem examinations performed on two of our patients (Cases 3 and 4). The photomicrograph shown in Figure 1 illustrates the extensive character of the renal arteriolar sclerosis in Case 4. From the clinical ex-

tients who suffered from both pyelonephritis and hypertension.

A detailed review of the clinical observations concerning the association of pyelonephritis and hypertension in patients studied in this hospital, however, led to the hypothesis that the hypertension might well be related to the local effect of the pyelonephritis rather than to the renal insufficiency encountered late in the disease. When this hypothesis was put to an empirical test by the removal of the infected kidney in a patient who suffered from unilateral pyelonephritis and hyper-



FIG. 2. PHOTOMICROGRAPH OF MICROSCOPIC SECTION PREPARED FROM THE KIDNEY REMOVED FROM CASE 5

Hematoxylin and eosin. Reduced from a magnification of 240 diameters. Note diffuse pyelonephritis. No thickening of arterioles comparable to that noted in Case 4 is demonstrable.

amination of the retinal and peripheral vessels it is probable that nephrosclerosis was present in addition to the pyelonephritis in Cases 1 and 2. It is clear that no conclusion may be drawn from our evidence in these four cases concerning the relative time of onset of the pyelonephritis and the nephrosclerosis or their relative importance in the production of hypertension. The same difficulty was encountered by Kimmelstiel and Wilson (11) when they attempted to decide whether they were dealing with primary "vascular" hypertension or with secondary "renal" hypertension in their pa-

tension (Case 5) it was found to be effective. The results in Dr. Barney's case (Case 6) lend further support to such a relation between the pyelonephritis and hypertension.

Pathological examination of the kidney removed from our patient, Case 5, showed in addition to severe pyelonephritis very early sclerosis of the renal arterioles. These vascular lesions were not sufficiently advanced or prominent to merit the term of nephrosclerosis as it is ordinarily understood. The photomicrograph of Figure 2 illustrates the absence in Case 5 of such a

renal arteriolar sclerosis as observed in Case 4. It is well known that chronic inflammatory processes of various kinds are accompanied by vascular changes in the involved areas. In considering the rôle of ischemia and infections in the production of hypertension, it is of interest that Parker and Weiss (15) observed arteriolar sclerosis in the lung in the presence of pulmonary congestion and infection. That obstruction to the flow of urine from one kidney may result in a rise in blood pressure has been shown by Bell and Pedersen (2). Goldblatt, Lynch, Hanzal, and Summerville (16), and Wood and Cash (17) have produced hypertension in dogs by partially obstructing the blood supply to one or both kidneys. Both possibilities may play a rôle in the production of the hypertension secondary to pyelonephritis.

The clinical significance of the observations reported here is clear. At the present time we have no explanation as to why some patients with pyelonephritis develop hypertension before renal insufficiency while some develop it only after the renal damage has become marked, and still others die in uremia with very little hypertension. This question merits and will receive further clinical and pathological study.

#### PROTOCOLS

*Case 1:* M. F. (Hospital Number 194,041), a girl of 9 years, came to the hospital September 1935 because of recurrent frontal headache and vomiting and urinary frequency. The family history was not contributory to the patient's illness. The past history showed her to have been a full term baby whose development was somewhat delayed. She had mumps and pertussis at 5 years of age and measles at 8 years. Ever since birth she had unusual frequency and nocturia. At 5 years of age she began to have severe headaches coming on every 2 to 3 months and lasting all day. At 7 years of age, vomiting began to accompany the headaches which became more frequent. During the past year she failed to gain and was fatigued very easily. Recently the headaches and vomiting occurred weekly. Four months before admission, she was found to have pus in her urine and was placed on a ketogenic diet without effect on the pyuria.

A physical examination revealed a thin, chronically ill, mentally alert girl. Both of the fundi showed albuminuric retinitis; there was edema of the optic discs, and the retinal arteries were narrowed, tortuous, and gave a light reflex. The heart was enlarged, a roentgenogram showing a cardiac diameter of 9.8 cm. and an internal diameter of the chest of 18.5 cm. The aortic arch was

broad. There was a blowing systolic murmur and marked accentuation of the aortic second sound. The peripheral arteries were thickened but not beaded. The systolic blood pressure was 280 and the diastolic 170 mm. Hg. The right kidney was palpable. The red blood cell count was 3,700,000 per cu. mm. The hemoglobin was 72 per cent. The serum calcium was 9.6 mgm. per cent and the serum inorganic phosphorus 3.3 mgm. per cent. The blood urea nitrogen was 35 mgm. per cent and the serum protein 6.3 grams per cent. An intramuscular phenolsulphonephthalein test showed 27 per cent excretion in 300 cc. of urine in 3 hours. The urea clearance was 17 per cent of normal. The urine showed from a very slight trace to a large trace of albumin and numerous white blood cells per high power field.

Two weeks after admission she had a hypertensive crisis, the blood pressure rising to a systolic of 250 and a diastolic of 210 mm. Hg. She became comatose, delirious, and had several convulsions. The urine became grossly bloody. A week later she appeared as she did at admission. Six weeks after admission the blood pressure rose to 260 mm. Hg systolic and 230 mm. Hg diastolic, and she had a generalized convulsion. Twelve days later a right splanchnic nerve resection was done, and after another 3 days the left splanchnic nerve was resected. Following the second operation she was very anemic and was given four transfusions. The anemia continued. Eight days later she became oliguric and developed a parotitis and pericardial friction rub and after two more days died in uremia with a blood nonprotein nitrogen of 187 mgm. per cent. Permission for an autopsy was not obtained.

*Case 2:* B. M. (Hospital Number 110,250), a girl of 8 years, entered the hospital in November 1931 because of recurrent attacks of headache, fever and vomiting throughout the previous 18 months. Frequent examination of the urine by the family doctor during this period had shown that it always contained pus.

The past history showed she had been a normal baby and had developed normally. She had had measles, pertussis, and chickenpox. Following tonsillitis at 3 years of age she had had a tonsillectomy and adenoidectomy. Immediately before the present illness she had had mumps.

The physical examination showed a well developed but small, undernourished, pale girl who appeared chronically ill. The significant positive findings follow. The retinal arteries showed increased light reflex, narrowing of the lumen and tortuosity. The disks were blurred and hyperemic. The heart sounds were loud and forceful. There was a soft systolic murmur with a marked accentuation of the aortic and pulmonic second sounds. Radial and brachial arteries were thickened. The systolic blood pressure was 210 mm. Hg and the diastolic 170 mm. Hg. On repeated examination the urine contained a very slight trace of albumin and 5 to 30 white blood cells per high power field in uncentrifuged specimens. On culture of the urine there was a growth of *B. coli*. The blood nonprotein nitrogen was 24 mgm. per cent. An intramuscular phenolsulphonephthalein test showed 50 per cent ex-

cretion in two hours. A urea clearance was 65 per cent of normal. A urine concentration test showed an inability to concentrate above 1.012.<sup>2</sup>

Two weeks after entry the headaches became more severe. The following day the patient had a hypertensive cerebral crisis. The blood pressure at this time was 240 mm. Hg systolic and 170 mm. Hg diastolic. The patient recovered from this cerebral accident, and the blood pressure in the course of the next week fell to 180 mm. Hg systolic and 150 mm. Hg diastolic. Before an examination of the urinary tract could be made the parents removed the child against our advice.

*Case 3:* R. E. (Hospital Number 107,386), a boy 7 years of age, entered the hospital in January 1933 with the chief complaint of severe headaches for several years and vomiting and convulsions during the past 9 months. He was a full term baby born following a normal labor. His early development was normal, except that he grew slowly, weighing 21 pounds at 2 years of age, 24 pounds at 3 years of age, and 32 pounds at 5 years of age. At 2 years of age he was found to have a marked phimosis which had caused pain on urination for the past 4 months. At this time there was some tenderness to pressure over the bladder, and the urine was found to contain a trace of albumin, but no white blood cells. A circumcision relieved the painful urination. He had pertussis at 2 years of age, had broken a wrist in a fall at 5 years of age, and one month before admission had received a blow over the left eye which had caused a hemorrhage into the eye which had been treated at the Boston City Hospital. He had had frequency and nocturia for several years. During the past 5 months the vomiting was frequently projectile. Two months before admission it was known that his systolic blood pressure was 220 mm. Hg.

Physical examination revealed a somewhat underdeveloped and undernourished boy weighing 37 pounds, who was mentally alert and in no apparent distress. The significant positive findings were as follows. Both retinas showed multiple scarring from old hemorrhages. The retinal arteries were narrow and the veins unusually tortuous. The disk margins were indistinct, and there was slight papilledema. The radial and brachial arteries were thickened. The heart was slightly enlarged to the left. The aortic second sound was greatly accentuated. The systolic blood pressure was 240 mm. Hg and the diastolic blood pressure, 190 mm. Hg. The liver was 3 cm. below the costal margin. The spleen was palpable. Repeated examination of the urine showed a slight trace of albumin but no sediment. The blood nonprotein nitrogen was 30 mgm. per cent. Serum calcium and phosphorus were normal. An intramuscular phenolsulphonephthalein test showed 32 per cent excretion in 2 hours. The urea clearance was 100 per cent of normal. A concentration test showed the specific gravity to be limited to 1.012.<sup>2</sup> Roentgenograms of the skull showed definite separation of the coronal and sagittal sutures indicating increased

intracranial pressure. Ventriculograms showed no localized distortion or filling defect. The very slight dilatation of the ventricles was consistent with a cerebral edema. The long bones were negative for lead. The red blood cells showed no stippling. The diagnosis of malignant hypertension of unknown etiology was made. The child was discharged to a convalescent home on February 28th with the same hypertension with which he entered. He died of cardiac failure August 9, 1933, without re-entering the hospital. An autopsy performed outside the hospital<sup>3</sup> showed the following: cardiac hypertrophy and dilatation, sclerosis of aorta, coronary and cerebral vessels, bilateral pyelonephritis and hydronephrosis, arteriolar nephrosclerosis, bilateral hydro-ureters, cystitis and dilatation of the bladder.

*Case 4:* R. W. (Hospital Number 143,483), an 11 year old boy, was admitted to the hospital October 14, 1930, because of severe recurrent attacks of frontal headache, nausea, and vomiting beginning abruptly 11 months ago. The attacks occurred about once a week and were frequently precipitated by emotional disturbances. In addition to these symptoms there had been severe upper abdominal pain and dizziness over the past month. The family history was not relevant. The past history showed the patient to have been born a healthy child following a normal labor. His development was normal. He was reported to have had measles at 2 months of age, pertussis at 3 years, and repeated attacks of tonsillitis up to 2 years ago, when his tonsils and adenoids were removed.

Physical examination revealed a poorly developed slightly underweight child. The blood pressure was 260 mm. Hg systolic and 180 mm. Hg diastolic. The peripheral arteries were thickened. After rest, the blood pressure varied from 210 to 150 systolic and 160 to 120 diastolic. There was an albuminuric retinitis with hemorrhages and exudate. The heart was not enlarged. Following the administration of 0.12 gram of sodium nitrite by mouth the blood pressure fell from 200 systolic and 150 diastolic to 155 systolic and 130 diastolic, the hands became cold and fingers and face cyanotic, and the brachial, cubital and radial arteries became softer. The urine contained from a very faint trace to a trace of albumin, moderate and varying numbers of white blood cells, and occasional hyaline and granular casts. The urea clearance was 88 per cent of normal and the specific gravity of the urine varied from 1.020 to 1.005.

One month after admission the pain in the left flank became severer, the temperature rose to 103° F., the pyuria became more pronounced, and there was local tenderness and fullness in the left kidney region. The white blood count rose to 22,000. It was at this time that the lower blood pressures were observed. An exploratory operation was deemed advisable. Through an incision running obliquely forward from the left side of the costovertebral angle the left kidney was exposed. The kidney was found to be markedly edematous. Toward

<sup>2</sup> We have noted such a discrepancy between the specific gravity and urea clearance tests in several of our patients with hypertension.

<sup>3</sup> We are indebted to Dr. Williams of the Rhode Island General Hospital for the autopsy report.

the lower pole there was a large amount of cellulitis and a small amount of pus. Nothing was felt in the position of the adrenal. A drain was inserted to the infected area and the wound closed in layers to the drain. Pus drained from the wound for 10 days. The drain was then removed, and the wound healed well. The urine continued to show a few white blood cells. The albuminuric retinitis had now disappeared. After another 10 days the blood pressure began gradually to increase until it had again reached 220 systolic and 175 diastolic. At this time the spinal fluid pressure was 350 mm. H<sub>2</sub>O. Intravenous pyelograms showed the dye to be well concentrated and bilateral enlarged renal pelves and blunted calyces. The renal function remained unchanged.

On January 2, 1931, a second exploratory operation was performed through a left rectus incision from the costal margin to umbilicus and a transverse incision from umbilicus to perpendicular line from anterior superior spine. The right side of the abdomen, right kidney, and suprarenal was explored and nothing abnormal was made out. The peritoneum over the left kidney was incised and retracted. There was a moderate amount of inflammation about the enlarged left kidney. The left adrenal appeared normal.

Following the operation, the blood pressure continued its gradual rise reaching 250 mm. Hg systolic and 180 mm. Hg diastolic. At this time he had a hypertensive crisis with convulsions, pallor of left optic disc, and loss of sight. His heart showed slight left sided hypertrophy and a systolic murmur was heard at the apex. He recovered, regained his vision, and improved sufficiently to be discharged home to his family physician. The urine still showed a trace of albumin, rare white and red blood cells, epithelial cells and granular casts. The blood non-protein nitrogen was not elevated, the red blood count was 4,900,000 and hemoglobin 80 per cent. The blood pressure was 230 mm. Hg systolic and 175 mm. Hg diastolic.

He was readmitted to the hospital April 17, 1931, with cardiac failure. The blood pressure was 140 mm. Hg systolic and 100 mm. Hg diastolic. The urea clearance of 79 per cent was normal. He had repeated attacks of severe abdominal pain and dyspnea relieved by obstructing the venous return from the lower extremities. The cardiac decompensation increased, and he died 10 days after readmission.

Postmortem examination (A-31-68) showed bilateral chronic pyelonephritis and marked nephrosclerosis. *Streptococcus hemolyticus* was recovered from the heart's blood and from exudate in the right pleural cavity, the pericardial cavity, and the peritoneal cavity. Of particular importance in regard to the clinical story were the findings in the heart and vascular system, and in the kidneys. The heart was considerably enlarged, more marked on the left than the right side. On microscopic examination, diffuse fibrosis of the myocardium was found. There was generalized atheromatosis of the aorta. The smaller blood vessels of the pancreas, spleen, and periadrenal tissues were particularly involved. There was thickening of both the intima and the media with

definite narrowing of the lumina of these vessels. Considerable perivascular fibrosis was also present.

The kidneys were not weighed but appeared definitely enlarged. They were firm in consistency and were enclosed in strongly adherent and thickened capsules to which the fibrosed periadrenal tissues were firmly attached. On section, the parenchyma was pale, yellowish brown in color and bulged into the line of incision. The cortex measured between 4 to 5 mm. in thickness, and was poorly differentiated from the medulla. No striations or glomeruli could be recognized on the cut surface. The small arteries stood out conspicuously because of their thickened walls. The pelves of both kidneys were slightly dilated. No exudate was present. The ureters were of uniform caliber and showed no inflammatory changes grossly.

Five blocks were selected from the kidneys and sections were stained by several methods including Mallory's connective tissue stain. The main features were severe infection and vascular lesions. There was a widespread chronic and acute inflammatory process with considerable replacement fibrosis and occasional areas of acute inflammation which amounted to abscess formation. In a few fields, areas of parenchyma were completely necrotic and were replaced by masses of polymorphonuclear cells. The connective tissue stroma was edematous and was infiltrated with polymorphonuclear cells in scattered areas. The tubules showed acute degenerative changes. The epithelial lining cells were swollen, granular, and often contained fat or colloid droplets. Within the lumens of the tubules albuminous precipitate, hyaline and granular casts and cylinders composed of polymorphonuclear leukocytes were found. The arterioles were markedly thickened, and the lumens were decreased in many cases to the point of complete obliteration. Often, the walls of the arterioles were arranged in concentric rings in the characteristic onion layer arrangement. No hyaline deposits were found in the walls of the arterioles. The glomeruli showed various degrees of hyalinization of the vascular tufts up to complete hyaline change. Swelling of the endothelial cells with vacuolization was frequently noted in the capillaries of the glomeruli. In occasional areas where the acute inflammatory process in the renal parenchyma was most marked, the tufts of the glomeruli were infiltrated with polymorphonuclear leukocytes, and inflammatory cells and fibrin were occasionally noted in the capsular spaces. Large numbers of glomeruli, however, retained their normal appearance. (See Figure 1.)

The sequence of events cannot be stated with certainty. There is definite evidence of both old and recent pyelonephritis. It is impossible to state whether the arteriolar lesions in the kidney and in other organs of the body came before or after the initial infection of the kidneys. The association of vascular lesions of this type and pyelonephritis has been noted on several occasions in our laboratory. It should be emphasized, however, that the vascular lesions are not confined to the kidneys; the small vessels in the pancreas, spleen, and adrenals were also involved. The enlargement of the heart and the myocarditis are probably secondary to the vascular disease.

Case 5: P. B. (Hospital Number 187,522), a boy of 7 years, entered the hospital in November 1934 because of hematuria 3 to 4 times a week for the past two months and occasional dysuria. The family history was noncontributory. He had had a normal infancy and always been well except for chickenpox at 4 months of age, mumps at 2 years and measles at 6 years. Physical examination showed a well developed and nourished boy with no abnormal findings. The blood pressure was 98 mm. Hg systolic and 50 mm. Hg diastolic. The urine contained a slight trace of albumin and was loaded with red blood cells. A roentgenogram of the abdomen showed a large solitary calculus at the lower pole of the right kidney. Intravenous pyelograms two days later showed the calculus in the region of the lower end of the right ureter. The right kidney pelvis was considerably dilated and the calyces were blunted. The right ureter was not outlined. The left kidney pelvis, calyces, and ureter were normal. The blood nonprotein nitrogen was 20 mgm. per cent. A phenolsulphonephthalein intramuscular test resulted in 30 per cent excretion in the first hour and 25 per cent excretion in the second hour. The following day Dr. W. E. Ladd exposed the lower end of the right ureter and removed the calculus. On analysis, 73 per cent of the dry material was ash composed of calcium oxalate, carbonate, and phosphate. Retrograde pyelography showed a markedly dilated right kidney pelvis with blunted calyces and a large tortuous right ureter. Culture of the urine from the left ureter and bladder gave no growth. The urine cleared, and the child was discharged to the outpatient department.

In January 1935, the urine contained many white blood cells and culture showed a growth of *B. coli*. In spite of hexamethylenamine, ammonium chloride and ketogenic diet therapy the pyuria and bacilluria continued. On April 5, a right nephrostomy was done by Dr. W. E. Ladd. At the lower pole of the kidney the fat was abnormally adherent, and there were several aberrant vessels, which did not, however, appear to compress the ureter. The pelvis of the kidney was greatly dilated and its wall thickened, and the ureter was markedly dilated and kinked on itself at the level of the lower pole of the kidney. An incision was made in the surface of the kidney pelvis, and catheter introduced down into the ureter. The kinking of the latter was partially relieved by freeing dense, fibrous tissue, and the catheter could then be passed without difficulty the whole length of the ureter. A silk purse string suture was then placed in the mid point of the convexity of the kidney, and by sharp and blunt dissection a hole was made through the cortex of the kidney into the pelvis. A large catheter was inserted through this and carried out through the original incision. The renal cortex was much thicker than anticipated.

On April 25, a phenolsulphonephthalein intramuscular test showed 15 per cent excretion from the nephrostomy drain in 32 cc. in the first hour and 10 per cent in 26 cc. the second hour. The catheter was removed April 30, the wound healed well, and the patient was discharged

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On August 7 he still had pyuria. He was readmitted to the hospital on August 13 because of vomiting and rapid pulse for 5 days. The temperature was normal. He was cyanotic and dyspneic with respirations 48 and pulse 140 per minute. The liver was enlarged to the umbilicus and there was dependent edema. The heart was large, the diameter measuring 13.5 cm. as compared to an internal diameter of the chest of 21.4 cm. Auscultation of the heart showed dropped beats. The blood pressure was 130 mm. Hg systolic and 100 mm. Hg diastolic. There had never been any history suggesting rheumatic fever or any acute infection other than the pyelonephritis. After digitalization the pulse rate returned to normal, the dropped beats disappeared, there was a marked diuresis with loss of edema, and the blood pressure rose to 160 mm. Hg systolic and 105 mm. Hg diastolic. Urine from the right kidney contained *B. coli* and pus, and showed a 3 per cent excretion of intramuscularly injected phenolsulphonephthalein in one hour, while the left kidney gave 16 per cent excretion and no organisms on culture. A urine concentration test gave a specific gravity as high as 1.024. The blood nonprotein nitrogen was 24 mgm. per cent. The sedimentation rate was normal. As there had been no hematuria or cylindruria characteristic of nephritis at any time during this admission and no drop in blood pressure with the diuresis, it was felt that an acute hemorrhagic nephritis on top of the pyelonephritis could not account for the elevated blood pressure. No source of infection other than the right pyelonephritis could be found. During the following seven weeks the blood pressure varied between 122 to 168 systolic and 90 to 110 diastolic. For the specific purpose of checking the hypertension and cardiac involvement, a right nephrectomy was done by Dr. W. E. Ladd on October 5. The ureter was found to be greatly dilated, tortuous, and to be covered with numerous firm fibrous adhesions. About two inches above the bladder, the ureter regained its normal appearance, so at this point it was clamped, ligated, and cut with cautery.

Examination of the right kidney and ureter showed the following. The gross specimen consisted of a right kidney measuring approximately  $10 \times 6 \times 3$  cm., surrounded by a moderate amount of deep orange-colored perirenal fat. The capsule was thick, opaque, and in certain areas markedly adherent to the kidney surface. The external surface was a diffuse yellowish-brown in color, stippled with closely placed deep red punctate areas. Over the inferior pole was a large, irregular, slightly depressed dull purplish-red area to which the capsule was markedly adherent. At the superior pole were smaller discrete similar areas averaging 0.3 cm. in diameter. The kidney was moderately firm but boggy and cut with ease revealing poorly defined cortical and medullary zones. The cortex was slightly swollen, measuring approximately 0.7 cm. in thickness, slightly irregular, generally pale yellowish-brown in color with faint pinkish-gray linear

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radiations. At the superior pole there was a dull, moist, purplish-red, wedge-shaped lesion with a deep red central area, extending to the tip of an inferior calyx. The medulla was moist, pale brownish-yellow in color with faint red linear radiations. The pyramids were pale yellowish-brown, smooth and moist. The pelves and calyces were greatly dilated with moist, rough granular mucosal surfaces, dirty bluish-yellow in color flecked with numerous small bright and dark red pin-point lesions. The ureter was dilated, measuring 2.8 cm. in circumference. It was redundant and kinked but patent. The mucosa was swollen, thickened, wrinkled, and dirty yellowish-gray in color. Numerous small irregular bright red pin-point lesions were scattered over the mucosal surface. The specimen was sectioned and fixed in Zenker's solution and 10 per cent Formalin solution.

Six sections of kidney and two sections of ureter were stained by various methods including Mallory's connective tissue stain. There was some thickening, edema, and lymphocytic infiltration of the capsule. The interstitial tissues were densely infiltrated by lymphocytes, polymorphonuclear leukocytes, and mononuclear cells. In occasional areas the renal parenchyma was replaced by polymorphonuclear leukocytes and lymphocytes. In other areas, lymphocytes were packed so densely that a suggestion of lymph follicle formation was noted. Inflammatory changes extended throughout the entire kidney and were prominent in the subpelvic tissues. The convoluted and collecting tubules were dilated, and the lining epithelium showed cloudy swelling, fragmentation of the cytoplasm, and in some areas flattening of the epithelium. Occasional tubules were filled with polymorphonuclear leukocytes. In many of the glomeruli there was increased prominence of the basement membrane both in the capillary loops and in the capsule itself. Many glomeruli were practically bloodless. The basement membrane was particularly thickened in the areas of greatest inflammatory infiltration, and the capsules of the glomeruli showed fibrosis and hyalinization. In a number of instances there were adhesions between the glomerular tufts and the capsule. Inflammatory cells in the glomeruli were lacking. Moderate thickening of both the media and intima of the smaller arterioles was noted. Edema, acute and chronic inflammation, and congestion were noted beneath the mucosa of the ureters. (See Figure 2.)

Postoperatively the blood pressure fell to 100 mm. Hg systolic and 70 mm. Hg diastolic, and the urine was clear. During the subsequent 20 months, examination at six outpatient visits have shown that the heart has been of normal size, the urine negative for pus and bacteria, the blood pressure never higher than 115 mm. Hg systolic and 75 mm. Hg diastolic, and the urea clearance within normal limits.

*Case 6: Dr. J. D. Barney's patient.* A girl 10 years of age with a history of pyuria of at least two years' duration was admitted to the hospital March 1937 with a bacilluria and a varying number of white blood cells in the urine and a hypertension of 190 mm. Hg systolic and 120 mm. Hg diastolic pressure. The blood nonprotein

nitrogen and the excretion of phenolsulphonephthalein were within normal limits. The day following the removal of the right kidney by Dr. Barney the blood pressure fell to 110 mm. Hg systolic and 70 diastolic and remained below that level during the subsequent stay in the hospital. Three months later, when the child was last seen, she appeared to be a healthy active child, and the blood pressure was 92 systolic and 60 diastolic. Dr. T. B. Mallory described the kidney as a small irregularly scarred kidney with dilated pelvis and slightly injected ureter, which on section showed a most marked thickening of the media and intima of the arterioles and an infiltration of cells in areas throughout the parenchyma and beneath the epithelium of the pelvis and several calyces.

I wish to acknowledge my indebtedness to Dr. Sidney Farber for the pathological descriptions of Cases 4 and 5 and for his coöperation in reviewing with me many cases of pyelonephritis, and to thank Dr. J. D. Barney and Dr. T. B. Mallory for permitting reference to their case.

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# ALLERGY AND DESENSITIZATION IN TUBERCULOSIS<sup>1</sup>

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(Received for publication July 6, 1937)

When guinea pigs are infected with tubercle bacilli they become allergic. Also, they develop a demonstrable degree of immunity. This, all workers in tuberculosis know. Indeed, the association of allergy and immunity is so close that many have postulated a necessary bond between them. In recent years, however, Rich (1) and several other observers (2) have done work which purports to show that allergy and immunity to tuberculosis have no obligate relation and can be separated—the animal may be deprived of its allergy, without suffering any loss of immunity. These investigators have desensitized allergic guinea pigs by repeated injections of tuberculin, have rendered them non-allergic, and have shown that such animals seem to be immune for several weeks (up to 65 days) after re-infection with virulent tubercle bacilli.

Our observations on this question embrace the use of approximately 500 guinea pigs in several series of experiments which have been of two varieties: (1) The desensitization of allergic animals and the study of their immunity and (2) the prevention of the development of allergy by daily injection of tuberculin<sup>2</sup> made prior to infection with virulent bacilli, and continued until the termination of the experiments. The development of tuberculosis in these latter, unsensitized animals was then compared with its development in the normal and the allergic control animals.

## *Tuberculosis in allergic-desensitized animals*

Briefly and without elaboration of the data, it may be stated that our first and larger group of experiments confirmed in part the impression gained by other workers that immunity persists

in desensitized animals for several weeks after re-infection. However, we learned that this protection is but temporary, for when such animals, continued in complete desensitization, live four months or more after re-infection, they no longer show such immunity but die of extensive tuberculosis of the lungs—this at a time when the allergic control animals show but an occasional, scattered tubercle here and there in the body. And when six months have passed after re-infection, all the desensitized animals have invariably died of tuberculosis, while most of the allergic animals remain relatively free from this disease.

## *Tuberculosis in normal-desensitized<sup>3</sup> animals*

The question then arose as to what would happen if normal guinea pigs were prevented from ever developing allergy after they had been infected, and our most significant results occurred in animals thus treated. The details in one series of these experiments may be given as representative. In this experiment 48 animals were used. Sixteen of these had had preliminary infection with R<sub>1</sub> (strain of low virulence) for three weeks. Sixteen were normal controls. The remaining sixteen were normal, but, in each one, daily injection of 1 cc. of Old Tuberculin had been started three days before all 48 were infected subcutaneously with a large dose<sup>4</sup> of virulent tubercle bacilli of the human type. The daily injections of tuberculin were continued in the third group. All animals were skin tested with tuberculin at the third, the sixth and the tenth week after infection. The previously allergic animals remained allergic and gave tuberculin reaction; the normal animals became allergic at the third week and remained so; the third group—the normal-desensitized group

<sup>1</sup> Presented in part before the meeting of the American Society for Clinical Investigation at Atlantic City, May 3, 1937.

<sup>2</sup> The tuberculin used in our experimental work was very kindly furnished by Parke, Davis & Co.

<sup>3</sup> "Normal-desensitized" may seem a contradiction of terms. However, it expresses better than any other phrase the actual status of these animals.

<sup>4</sup> Four-tenths milligram moist weight inoculated in 0.4 cc. saline in each pig.

WEEK AFTER VIRULENT INFECTION	1	2	3	4	5	6	7	8	9	10	11	12
NORMAL				■	■	■	■	■	■	■		■
NORMAL-DESENSITIZED	■			■	■	■		■	■	■	■	
ALLERGIC		■							■	■		■

■ DEATH DUE TO NON-SPECIFIC CAUSES  
 ■ MODERATE TUBERCULOSIS  
 ■ EXTENSIVE TUBERCULOSIS  
 S SACRIFICED

FIG. 1. MORTALITY RECORD FOR THE THREE GROUPS OF ANIMALS

The animals died in the week indicated except that in the 12th week two animals were sacrificed.

which received daily injections of tuberculin—never developed allergy.

Briefly stated, the results were as follows for the normal control group (Figure 1). In the fourth, fifth and sixth week following infection, six animals died of nonspecific pneumonia, but each one had tubercles in the lungs. From the seventh to the tenth week, nine of the animals succumbed to tuberculosis. The last one was killed in the twelfth week and was found to have miliary tuberculosis.

Of the 16 animals in the normal-desensitized group, one died in the first week and two in the fourth week with nonspecific pneumonia. In the fifth and sixth weeks 4 animals died due to non-specific pneumonia, but they also showed tubercles in the lungs. All animals in this group

which lived as long as eight weeks after infection were found to show, at autopsy, an unusual degree of tuberculous pneumonia, characterized by absence of ordinary tubercles and by marked increase in the volume of the lungs, which were almost completely hepatized by the pneumonic process, (Figure 2). Smears made from the cut surface of these lungs showed innumerable acid fast bacilli (Figure 3).

Of the 16 guinea pigs in the allergic group, two died in the second week after reinfection, two in the ninth and three in the tenth, all with non-specific pneumonia, but the latter 5 animals showed an occasional tubercle. One was killed in the twelfth week and showed scattered tubercles in the viscera. Eight remained alive and well after twelve weeks.

It may be stated further that this striking and extensive involvement of the lung is but a part of the characteristic picture, for, as the tuberculous process develops in the lungs of these animals, the disease spares the other viscera so that the spleen and liver remain of practically normal size and appearance.

Whatever else may be deduced from the factors contributing to these results, it is clear at least that normal-desensitized (non-allergic) guinea pigs provide fertile soil for the tubercle bacillus; that such animals, without allergy, are without immunity.



FIG. 2. LUNGS FROM ANIMALS REPRESENTATIVE OF THE THREE GROUPS, IN THE THIRD MONTH AFTER THE VIRULENT INFECTION

To the left, lung from an allergic, reinfected pig. No definite tubercles can be identified in the gross. Middle, lung from normal control animal. Scattered tubercles can be seen. Right, lung from normal-desensitized pig. All lobes are distended with tuberculous bronchopneumonia. Smears from these lungs show innumerable acid-fast bacilli. Photograph  $\frac{2}{3}$  natural size.

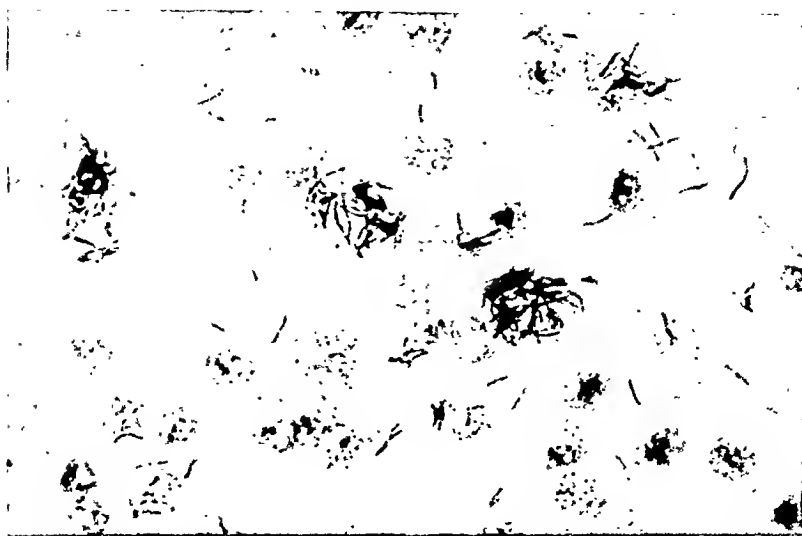


FIG. 3. IMPRESSION SMEAR FROM LUNG OF DESENSITIZED GUINEA PIG SHOWING THE GREAT NUMBER OF ACID-FAST BACILLI

Ziehl-Neelsen stain. Photograph taken with blue light.  $\times 1200$ .

#### SUMMARY

(1) Allergic-desensitized guinea pigs experience a delay of several weeks (after reinfection) in the development of tuberculosis. This delay has been mistaken for retained immunity.

(2) Animals which have been prevented from developing allergy by injection of tuberculin are unusually susceptible to tuberculosis and develop what is probably the most marked degree of tuberculous pneumonia yet produced in experimental animals.

(3) These observations indicate that it is unsafe as yet to conclude that the phenomena of al-

lergy are an unessential part of the mechanism of defense against tuberculosis.

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# RELATION OF SERUM CALCIUM TO SERUM ALBUMIN AND GLOBULINS

By ALEXANDER B. GUTMAN AND ETHEL BENEDICT GUTMAN

(From the Department of Medicine, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital, New York City)

(Received for publication July 9, 1937)

In 1923, Salvesen and Linder (1) pointed out that hypoproteinemia in the nephrotic type of Bright's disease is associated with hypocalcemia, the calcium content of the serum tending to parallel the total serum protein level. Marrack and Thacker (2) and Hastings, Murray and Sendroy (3) subsequently expressed this proportionality between total calcium and total protein more precisely in the form of an empirical regression equation. By plotting the calcium content of body fluids of low or normal protein content as ordinates against the respective total protein values, they showed that the points so obtained approximate a straight line which has a positive slope and intersects the "y" (Ca) axis at a point above the origin. Such a linear relation, since confirmed in nephrotic and normal sera by others (4, 5, 6), may be expressed by the general regression equation (5):

$$\text{Total Ca} = m \cdot \text{total protein} + b, \quad \text{I}$$

where  $m$ , the slope of the line, is a constant which defines the amount of calcium bound per unit total protein and  $b$ , the intercept on the "y" axis, is a constant which defines the amount of calcium not bound to protein.

Though derived from clinical data, and purely empirical, Equation I is in accord with deductions drawn from dialysis and ultrafiltration experiments. These indicate that calcium present in serum is partly in a non-diffusible, partly in a diffusible state. At physiological concentrations of  $\text{Ca}^{++}$ ,  $\text{PO}_4^{--}$  and  $\text{H}^+$ , the non-diffusible calcium fraction appears to be protein-bound calcium; and values obtained for the diffusible calcium fraction, which is composed largely of  $\text{Ca}^{++}$  (7, 8), are in satisfactory agreement with values obtained for  $b$ . McLean and Hastings (5) have shown, further, that Equation I may be derived from a general mass law equation for the dissociation of

calcium proteinate where the protein molecules are assumed to be composed of a series of negatively charged divalent ions:

$$\frac{[\text{Ca}^{++}] \cdot [\text{Prot.}^{--}]}{[\text{Ca Prot.}]} = K. \quad \text{II}$$

Substituting  $[\text{Total Prot.}] - [\text{Ca Prot.}]$  for  $[\text{Prot.}^{--}]$  and simplifying, we obtain:

$$\frac{[\text{Ca}^{++}] \cdot [\text{Total Prot.}]}{[\text{Ca Prot.}]} = K + [\text{Ca}^{++}].$$

Substituting  $[\text{Total Ca}] - [\text{Ca}^{++}]$  for  $[\text{Ca Prot.}]$  and dividing both sides of the equation by  $[\text{Ca}^{++}]$ , we obtain:

$$\frac{[\text{Total Prot.}]}{[\text{Total Ca}] - [\text{Ca}^{++}]} = \frac{K}{[\text{Ca}^{++}]} + 1.$$

For the special case where the concentration of  $\text{Ca}^{++}$  is constant (and the experimental data used in deriving Equation I are restricted by definition to that condition), the constant  $b$  may be substituted for  $[\text{Ca}^{++}]$ . Designating the relation of constants  $\frac{K}{b} + 1$  by the reciprocal of the constant  $m$ , we obtain:

$$\frac{[\text{Total Prot.}]}{[\text{Total Ca}] - b} = \frac{1}{m}.$$

which solved for  $[\text{Total Ca}]$  gives Equation I.

Equation I does not apply if there is a primary disturbance in calcium metabolism (3) nor in the presence of hyperphosphatemia (1, 4). But apart from these restrictions, this equation seems so well supported by both empirical and theoretical evidence that it has come to be regarded as a generally valid expression of the relation between total calcium and total serum protein.

For example, it is inferred—as follows from Equation I—that elevated calcium values are to be expected in association with hyperproteinemia. Since a significant proportion (almost half) of the total calcium in normal serum is bound to





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For example, it is inferred—as follows from Equation I—that elevated calcium values are to be expected in association with hyperproteinemia. Since a significant proportion (almost half) of the total calcium in normal serum is bound to

protein, it does seem to follow that with increased protein content there would be an increase in calcium bound to protein and, consequently, a rise in the total calcium content of the serum; hyperproteinemia being regarded in this sense as "a cause of" or "responsible for" hypercalcemia. A number of cases of multiple myeloma have been described in which hyperproteinemia was, in fact, associated with hypercalcemia. And in such cases, a definite increase in the non-diffusible (or protein-bound) calcium fraction could be demonstrated by ultrafiltration—a result in accord with the implications of Equation I.

Despite this considerable body of supporting evidence, however, empirical equations of the general form of Equation I should be regarded, as Peters and Van Slyke (9) have pointed out, only as "rough first approximations" of the relation of calcium to protein in serum. It has often been noted that when, as in hepatic cirrhosis (10), decreased serum albumin is associated with increased serum globulin, the serum calcium level seems to parallel the *albumin* rather than the total serum protein content. Such observations led Schmidt and Greenberg (11) to conclude that "because the protein bound calcium probably is very largely united to the albumin rather than to the total protein, the total protein content of the serum can only give an inadequate representation."

Our own data indicate that in a variety of diseases presenting hyperglobulinemia, there is a serious discrepancy between observed serum calcium and that calculated by formulae based upon Equation I (6, 12). It would appear that Equation I in its present form is not generally valid (within the limitations prescribed above), as has been assumed. Analysis of our data suggests further that a more satisfactory approximation could be effected by an expression relating calcium specifically to the several protein fractions of which the total serum protein is composed. A definitive equation of this kind is not attainable for the present because of the prevailing uncertainty regarding the serum protein fractions and their calcium-binding properties under the conditions existing in serum. By graphic and statistical analysis of our data, however, we derived an equation relating total calcium to serum albumin and two arbitrarily defined serum globulin fractions. This equation appears to give better agreement between

observed and calculated serum calcium over a wide range of variation in serum proteins than do equations relating total calcium to total protein, to albumin and total globulin, or to albumin alone.

#### MATERIAL AND METHODS

Our data (Table I) include 27 observations on 21 cases of the nephrotic syndrome, with low albumin and normal or somewhat decreased globulin levels; 20 observations on 15 normal subjects; 50 observations on 39 cases of lymphogranuloma inguinale, 37 sera containing more than 8.0 per cent total protein; 25 observations on 20 miscellaneous cases with hyperproteinemia not due to lymphogranuloma inguinale, multiple myeloma or hepatic cirrhosis; and 42 observations on 28 cases of hepatic cirrhosis, with low or normal albumin and normal or high globulin levels. With the exception of cases of extreme dehydration, which were not available for study, the data may be regarded as illustrative of the types of changes in serum proteins occurring in disease.

For reasons stated in the text, conditions in which the concentration of  $\text{Ca}^{++}$  per unit serum water could not be assumed to be within normal limits were excluded: Cases with a primary disturbance in calcium metabolism (including multiple myeloma); cases with hyperphosphatemia; and cases with hypoproteinemia due to malnutrition or occurring in terminal stages of wasting diseases. Apart from these restrictions, and the inclusion of only a few representative normal values, the data are unselected.

Serum calcium was determined by the Clark and Collip modification of the Kramer and Tisdall method (30). Serum protein was determined by difference, total nitrogen by the Kjeldahl technique, and nonprotein nitrogen by Folin's method with nesslerization (31). Albumin and the globulin fractions were estimated by Howe's method (32), nitrogen being determined by the micro-Kjeldahl technique and titration. Inorganic phosphorus was determined by the method of Kuttner and Lichtenstein as modified by A. Bodansky (33). All determinations were carried out in duplicate, except some calcium analyses, when insufficient serum was available.

In view of the wide range in total protein content of our sera, the concentrations of all relevant solutes were expressed in terms of serum  $\text{H}_2\text{O}$ . This involves a correction:

$$C_w = \frac{C_s}{W_s} \cdot 100,$$

where

$C_w$  = concentration of solute per unit weight of serum  $\text{H}_2\text{O}$ .

$C_s$  = concentration of solute per unit volume of serum.  
 $W_s$  = grams of  $\text{H}_2\text{O}$  per 100 cc. serum.

$W_s$  was calculated by the McLean and Hastings' formula (5):

$$W_s = 99.0 - 0.75P_s,$$

where

$P_s$  = grams of total protein per 100 cc. serum,  
0.75 = the Svedberg and Sjögren factor for specific  
molecular volume of serum protein

and 1 per cent of serum volume is assumed to be occupied  
by solutes other than protein.

A quotient nomogram (Figure 1), which proved useful for rapid conversion of large groups of data and for checking values calculated in the usual manner, was constructed on the basis of the formula:

$$\log x = \log y + \text{colog } z$$

where

$x = C_w$  as defined above,

$y = C_s$  as defined above,

$z = W_s$  as defined above  $\div 100$ .

Column  $P_s$  represents the observed serum protein content in grams per 100 cc. serum. The columns  $C_s$  and  $C'_s$  represent the observed concentration per 100 cc. serum of that solute which is to be expressed in terms of serum water;  $C_s$  being scaled to cover the range 1.5 to 3.0 and 3.0 to 6.0; the scale of  $C'_s$  reading from 1.0 to 1.5 and from 6.0 to 10.0.<sup>1</sup> A straight line connecting the point on the  $P_s$  column corresponding to the observed total protein content of the serum, with the point on the  $C_s$  or  $C'_s$  column corresponding to the observed concentration of solute in serum, will intersect the adjacent  $C_w$  or the respective  $C'_w$  column at a point representing the desired concentration of solute in serum water.

The nomogram is applicable to sera containing 3.0 to 12.0 grams of total protein per 100 cc. serum. It gives results accurate to the third significant figure over the range of variation in serum electrolytes (expressed in milligrams respectively grams, or in milliequivalents).

## RESULTS

*Relation of total serum calcium to total serum protein in the nephrotic syndrome and in normal subjects.* In Figure 2, we have plotted values for total serum calcium against the respective total protein content of sera obtained from patients with the nephrotic syndrome. To show the trend more clearly, 4 observations on 4 cases of "healed nephrosis" and 15 representative results on normal subjects are included. The points, indicated by hollow dots, show a positive linear correlation which is in good agreement with Equation I. The straight line shown in Figure 2 was not drawn through our points but represents Equation I where  $m = 0.75$  and  $b = 5.6$ , total protein being expressed in grams, total calcium in milligrams

<sup>1</sup> To convert solute concentrations greater than 10.0, move the decimal point to conform with scales of the  $C_s$  or  $C'_s$  column and the respective  $C_w$  or  $C'_w$  column.

per 100 grams serum water. These values for  $m$  and  $b$  are the means of the several constants obtained empirically by various investigators, as calculated by McLean and Hastings (5).

Our results, therefore, are in accord with the findings in the literature with respect to the direct proportionality between total serum calcium and total serum protein in the nephrotic syndrome and in normal subjects. As a corollary, it may be inferred that the standard methods employed in determining calcium and protein give results in our hands which are consistent with those obtained by others; and consequently the discrepancies encountered in the diseases now to be considered are not due to differences in technique.

*Relation of total serum calcium to total serum protein in lymphogranuloma inguinale.* As was pointed out elsewhere (13, 14), many cases of lymphogranuloma inguinale present hyperproteinemia, the total protein reaching levels as high as 11 grams per 100 cc. serum. Hyperproteinemia in lymphogranuloma inguinale is not, however, associated with hypercalcemia (13, 14). The points obtained by plotting total calcium against the respective total serum protein in our cases (Figure 2) do not fall along the straight line corresponding to Equation I. The trend shown by these points is approximately parallel to the "x" axis. It would appear, therefore, that the linear relation between total serum calcium and total serum protein observed in hypoproteinemia does not obtain in hyperproteinemia due to lymphogranuloma inguinale.

*Relation of total serum calcium to total serum protein in miscellaneous diseases presenting hyperproteinemia.* This discrepant relation is not peculiar to hyperproteinemia occurring in lymphogranuloma inguinale. In Figure 3, we illustrate the distribution of points obtained by plotting total calcium against total serum protein in 20 miscellaneous cases presenting hyperproteinemia not due to lymphogranuloma inguinale, multiple myeloma or hepatic cirrhosis. This group is composed chiefly of various infections. In some instances, no diagnosis could be established. In several instances, lymphogranuloma inguinale was suspected clinically, but the Frei test was negative.

As in lymphogranuloma inguinale, the points do not fall along a straight line corresponding to Equation I but show a trend approximately paral-

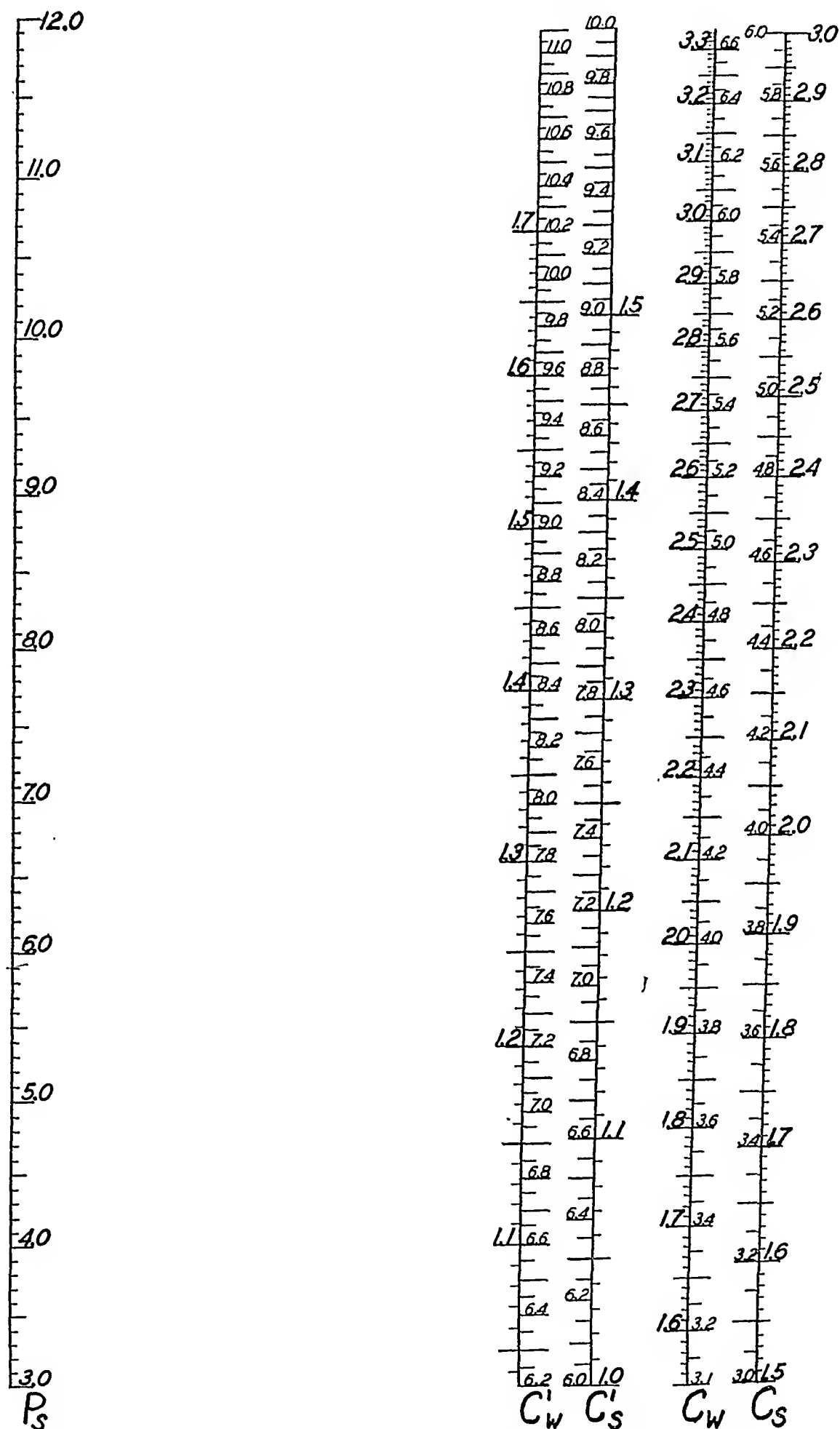


FIG. 1. NOMOGRAM FOR CONVERTING SOLUTE CONCENTRATIONS PER 100 CC. SERUM TO SOLUTE CONCENTRATIONS PER 100 GRAMS SERUM  $H_2O$

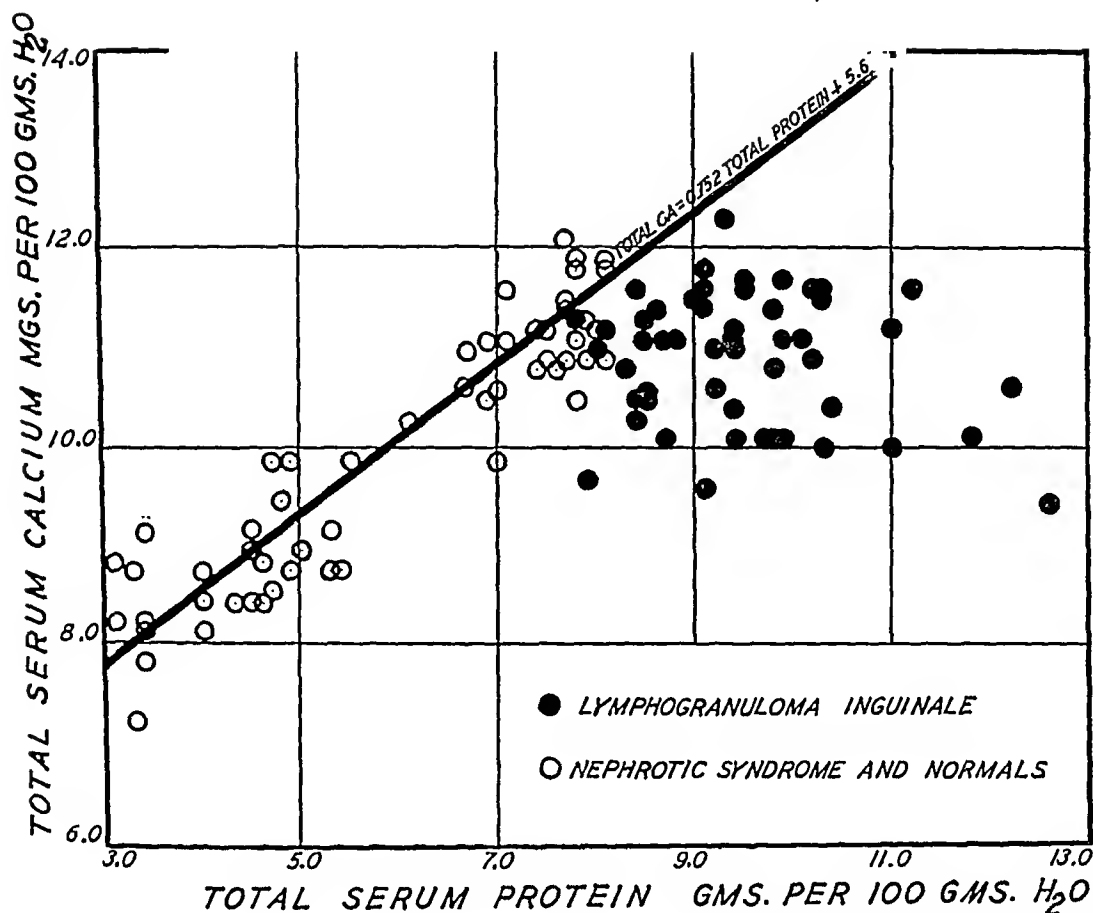


FIG. 2. RELATION OF TOTAL CALCIUM TO THE RESPECTIVE TOTAL PROTEIN CONTENT OF SERA IN THE NEPHROTIC SYNDROME AND IN NORMAL SUBJECTS, SHOWING A DIRECT PROPORTIONALITY BETWEEN THESE TWO CONSTITUENTS

This proportionality does not hold in hyperproteinemia due to lymphogranuloma inguinale.

rel to the "x" axis. Our results (Table I and Figure 3) indicate that, contrary to the implications of Equation I, the total serum calcium does not rise in hyperproteinemia but is maintained at normal levels. (This generalization does not apply to relative increases in serum proteins and other solutes occurring in extreme dehydration; or to conditions like multiple myeloma where there is a primary disturbance in calcium metabolism.)

*Relation of total serum calcium to total serum protein in hepatic cirrhosis.* Figure 3 also shows the distribution of 42 points obtained by plotting total calcium against the respective total serum protein content in cases of hepatic cirrhosis, in most instances of the Laennec type. The serum albumin may be considerably decreased in cirrho-

sis of the liver, as is well known, and some cases also show varying degrees of hyperglobulinemia (Table I).

The points representing our cases of hepatic cirrhosis do not fall along the straight line corresponding to Equation I. The discrepant relation of the 164 observations plotted in Figure 3 indicates that Equation I is not generally valid over the range of variation in serum proteins, as has been assumed.

*Calcium-protein relation in multiple myeloma, hyperphosphatemia and malnutritional hypoproteinemia; grounds for exclusion of these conditions.* It has been known for some time (15) that in multiple myeloma hyperproteinemia and hypercalcemia may co-exist. Of 75 published

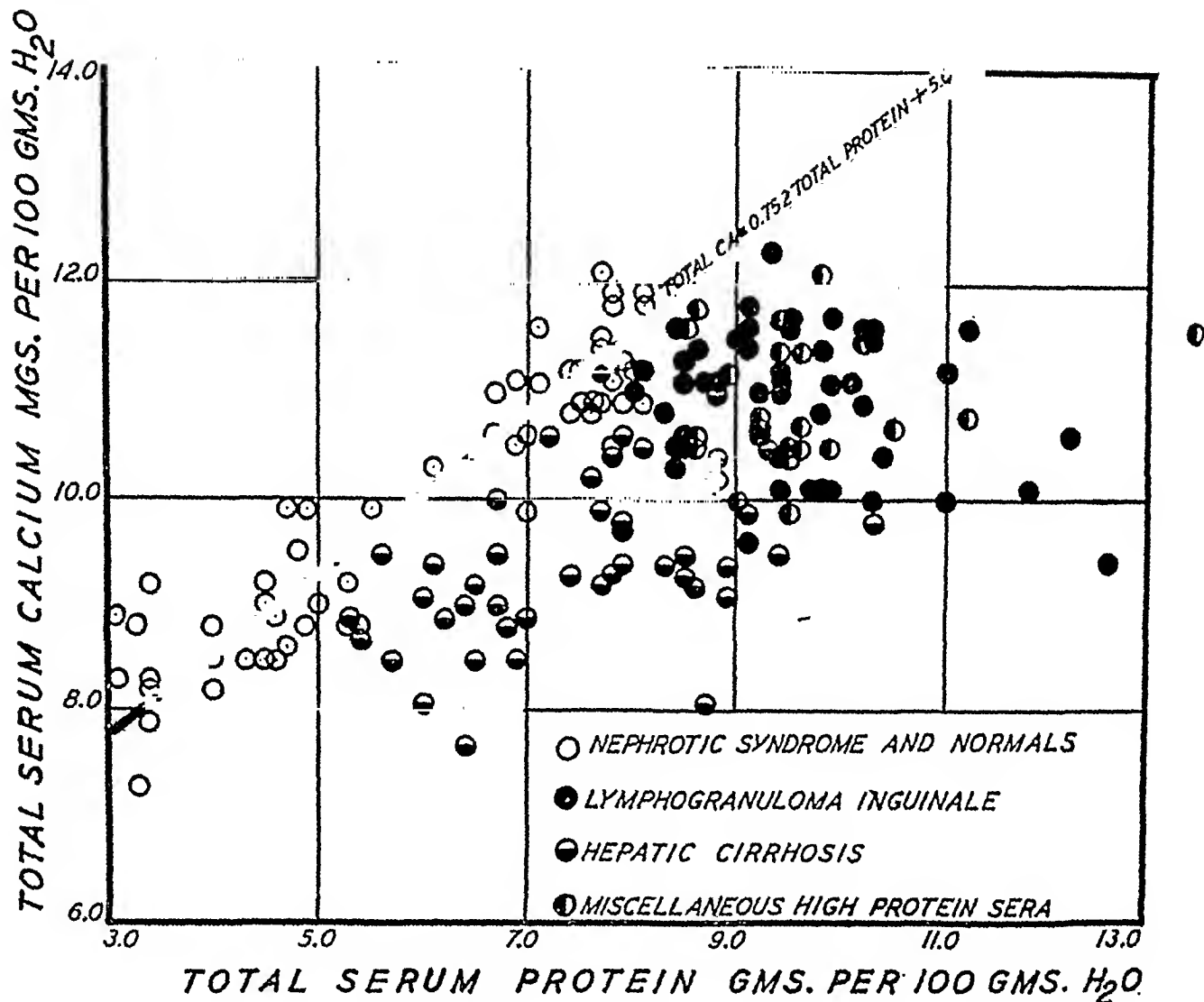


FIG. 3. TOTAL CALCIUM IS PLOTTED AGAINST TOTAL PROTEIN IN 164 SERA PRESENTING VARIOUS CHANGES IN SERUM PROTEINS

There is no linear relation between total calcium and total protein.

cases of multiple myeloma in which both protein and calcium were determined (including 15 of our own), hyperproteinemia occurred in 43 of which 29 also presented hypercalcemia. The co-existence of hyperproteinemia and hypercalcemia in multiple myeloma has been regarded as evidence for the validity of Equation I in hyperproteinemia. But when total calcium is plotted against the respective total serum protein found in published cases of multiple myeloma (Figure 4), it is evident that the points so obtained do not fall along the line corresponding to Equation I. In fact, no linear relation can be made out between total calcium and total serum proteins.

Multiple myeloma is a disease characterized by extensive bone destruction and should be classified with conditions presenting a primary dis-

turbance in calcium metabolism. Since, as stated at the outset, Equation I applies only where there is no primary disturbance in calcium metabolism, we regard discussion of the conformity or lack of conformity of such cases to Equation I as irrelevant; and feel justified in omitting our cases of multiple myeloma, by definition, from the data subjected to analysis.

The hypercalcemia observed in many cases of multiple myeloma (18) may well be due, not to hyperproteinemia (which may not be present in association with the hypercalcemia), but to the complication of co-existent bone destruction by neoplastic tissue; like the hypercalcemia found occasionally with metastatic osteolytic carcinoma, in which serum proteins are normal or low (18, 19). That the majority of cases of multiple myeloma

TABLE I

Total protein, albumin and calcium content of 144 sera obtained from patients with diseases affecting the serum proteins; and of 20 normal sera

Total protein	Albumin	Total calcium	Total protein	Albumin	Total calcium
grams per 100 cc. serum	grams per 100 cc. serum	mgm. per 100 cc. serum	grams per 100 cc. serum	grams per 100 cc. serum	mgm. per 100 cc. serum
LYMPHOGRANULOMA INGUINALE			HYPERPROTEINEMIA OF MISCELLANEOUS ORIGIN—Cont.		
11.4	2.6	8.5	8.9	4.2	9.7
11.1	3.3	9.6	8.8	3.6	9.7
10.7	2.9	9.2	8.8	3.5	9.6
10.3	3.1	10.6	8.8	3.0	9.1
10.1	3.5	10.3	8.7	4.6	10.8
10.1	3.3	9.1	8.7	4.0	10.5
9.6	3.6	9.5	8.5	4.0	10.0
9.5	3.4	10.7	8.5	4.0	9.9
9.5	3.1	10.6	8.3	3.8	9.2
9.4	3.6	10.0	8.2	4.6	10.4
9.4	3.4	10.7	8.2	3.3	9.5
9.3	3.3	10.2	8.0	4.6	9.8
9.1	4.0	10.2	8.0	4.4	11.1
9.1	3.5	10.8	8.0	4.3	11.0
9.1	3.1	9.4	8.0	4.3	9.9
9.0	3.8	9.9	7.9	3.9	10.8
9.0	3.6	10.5			
9.0	3.5	9.3			
8.8	3.8	10.7			
8.8	3.2	10.8			
8.7	3.5	10.2			
8.7	3.4	9.3			
8.7	3.0	9.3			
8.6	4.0	11.3			
8.6	3.6	10.1			
8.5	3.8	10.4			
8.5	3.7	10.2			
8.5	3.5	9.6			
8.4	4.2	10.8			
8.4	4.0	10.6			
8.4	3.9	9.8			
8.4	3.2	10.9			
8.4	2.3	8.9			
8.3	4.1	10.7			
8.2	3.8	10.3			
8.1	3.9	10.3			
8.1	3.3	9.4			
8.0	3.6	10.6			
7.9	4.3	10.3			
7.9	4.2	9.8			
7.9	4.1	10.5			
7.9	3.7	9.8			
7.8	4.7	10.8			
7.8	4.7	9.6			
7.8	3.9	9.8			
7.7	3.8	10.1			
7.6	4.3	10.4			
7.5	3.3	10.3			
7.4	2.9	9.1			
7.3	3.1	10.6			
HYPERPROTEINEMIA OF MISCELLANEOUS ORIGIN			CIRRHOSIS OF THE LIVER		
12.1	2.9	10.4	9.5	2.2	9.0
10.3	4.3	9.9	8.7	2.4	8.8
9.6	3.7	9.8	8.6	4.1	9.7
9.5	3.2	9.2	8.4	2.4	9.2
9.4	4.6	10.6	8.2	3.0	10.5
9.1	4.2	9.7	8.2	2.2	8.7
9.0	3.4	11.2	8.2	2.0	8.4
8.9	4.5	10.5	8.1	3.8	10.2
8.9	4.3	9.9	8.1	1.9	7.5
			8.0	2.9	8.6
			7.9	2.4	8.8
			7.9	2.1	8.7
			7.7	3.1	9.7
			7.6	3.2	9.8
			7.4	4.1	9.9
			7.4	2.1	8.8
			7.4	1.8	9.2
			7.3	4.2	9.7
			7.3	2.4	8.7
			7.2	4.5	10.5
			7.2	2.2	9.3
			7.2	1.7	8.6
			7.1	1.7	9.6
			6.9	2.4	8.7
			6.8	4.2	10.0
			6.6	1.8	8.4
			6.5	2.1	8.0
			6.4	2.9	8.3
			6.3	3.5	9.4
			6.3	3.4	9.0
			6.3	2.2	8.5
			6.1	3.2	8.7
			6.1	1.8	8.9
			6.0	2.0	8.5
			6.0	1.6	7.3
			5.9	2.7	8.4
			5.8	1.8	8.9
			5.7	3.7	8.6
			5.7	1.7	7.7
			5.4	1.9	8.1
			5.3	2.3	9.0
			5.0	1.5	8.5

TABLE I—*Continued*

Total protein	Albumin	Total calcium	Total protein	Albumin	Total calcium
grams per 100 cc. serum	grams per 100 cc. serum	mgm. per 100 cc. serum	grams per 100 cc. serum	grams per 100 cc. serum	mgm. per 100 cc. serum
"HEALED" NEPHROSIS			NEPHROTIC SYNDROME— <i>Continued</i>		
7.1	4.6	10.1	3.3	1.5	8.0
6.7	4.4	10.4	3.2	1.3	8.1
6.5	4.6	10.4	3.2	1.1	7.1
6.3	3.9	10.4			
NEPHROTIC SYNDROME			NORMAL SUBJECTS		
5.8	3.2	9.8	7.6	4.9	10.9
5.2	3.0	9.4	7.6	4.4	11.0
5.1	2.3	8.4	7.6	3.8	10.2
5.0	2.8	8.8	7.5	5.1	10.5
5.0	1.8	8.4	7.4	5.0	10.2
4.8	2.5	8.6	7.4	4.5	10.6
4.7	2.5	9.5	7.3	5.1	11.0
4.7	2.1	8.4	7.3	4.9	9.9
4.5	3.4	8.2	7.3	4.8	11.0
4.5	2.4	9.5	7.3	4.8	10.5
4.3	2.0	8.6	7.2	5.0	11.3
4.3	2.0	8.1	7.2	4.9	10.8
4.1	2.2	8.1	7.2	4.8	10.7
3.8	1.9	8.2	7.1	5.2	10.1
3.8	1.8	8.5	7.0	4.7	10.5
3.8	1.7	7.9	6.9	4.6	10.5
3.8	1.5	8.2	6.7	4.6	10.9
3.8	1.3	7.5	6.6	4.5	9.3
3.3	2.0	8.9	6.5	4.0	9.9
3.3	1.6	8.0	6.3	4.5	10.0

presenting hyperproteinemia also exhibit hypercalcemia may mean only that myelomatosis severe enough to cause hyperproteinemia is likely to be extensive enough to produce widespread skeletal damage, resulting in hypercalcemia. An absolute increase in protein-bound calcium demonstrated by ultrafiltration in some cases of multiple myeloma occurs, apparently, only in cases presenting hypercalcemia, and then irrespective of whether or not the serum protein content is increased (6). We have suggested elsewhere (6) that this increase in protein-bound calcium is a *result*, not the cause of the hypercalcemia; a result of the influx of  $\text{Ca}^{++}$  caused by bone destruction, with re-establishment of equilibrium between these two fractions at higher levels of both ionized and protein-bound calcium. Moreover, it seems likely that, for reasons stated later, most of the increased protein-bound calcium in multiple myeloma presenting both hyperproteinemia and hypercalcemia is calcium bound by *albumin*; and little calcium is

bound by the euglobulin increment usually responsible for the hyperproteinemia.

Cases of hyperphosphatemia are excluded because hyperphosphatemia, as is well known, depresses the total calcium content of the blood by mechanisms not directly dependent upon the serum proteins. The presence of hyperphosphatemia lowers the  $\text{Ca}^{++}$  concentration and disturbs the equilibrium between  $\text{Ca}^{++}$  and protein-bound calcium, apparently in part, at least, through the formation of non-diffusible (colloidal) calcium phosphate complexes. Peters and Eiserson (4) have suggested an empirical equation relating calcium to total protein and inorganic phosphorus. We have not attempted to formulate such a more general equation from our data, however, but have included in Table I only sera containing 2.5 to 5.0 mgm. inorganic phosphorus per 100 cc. serum.

In our cases of malnutritional hypoproteinemia, points obtained by plotting total calcium against



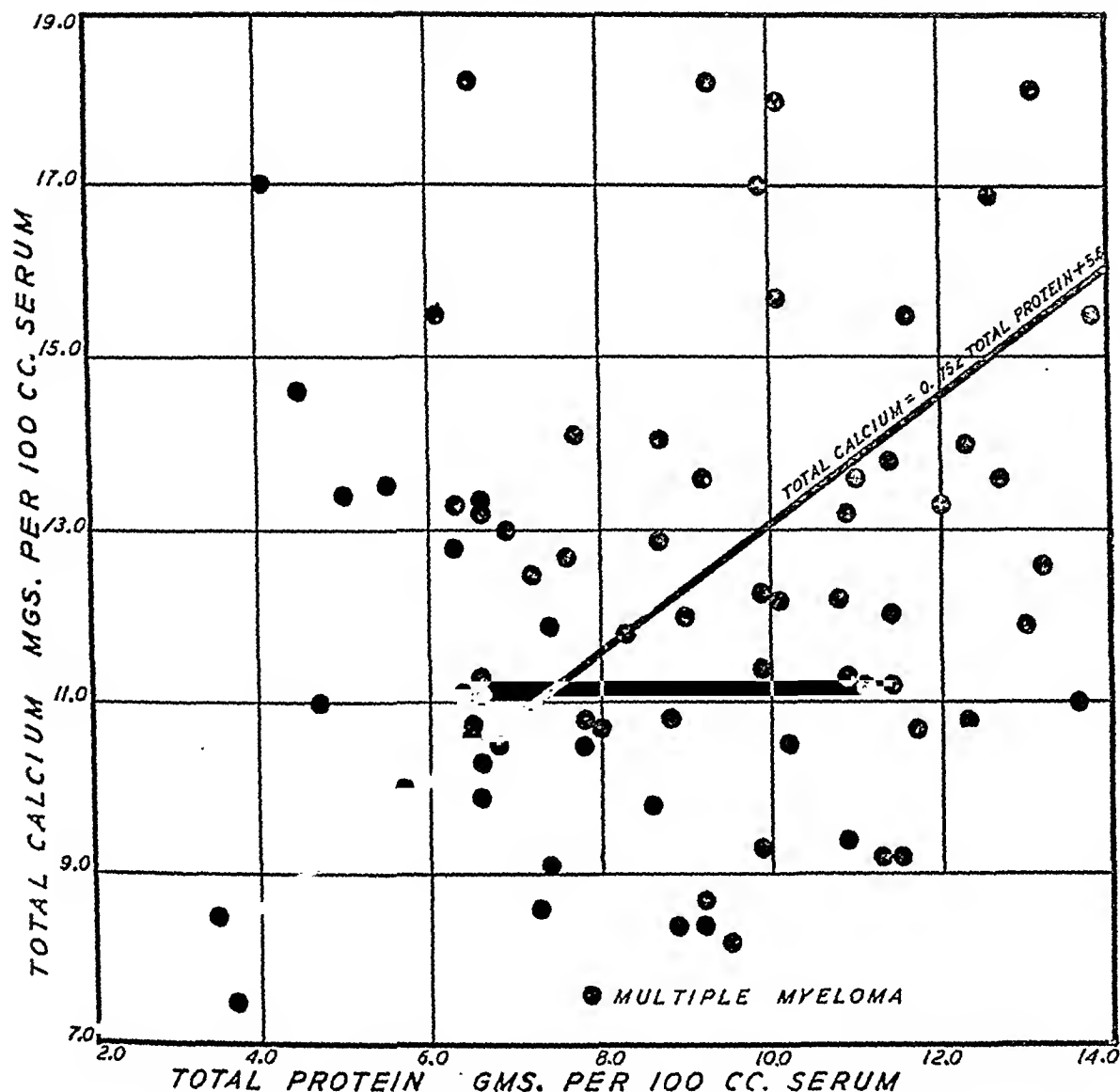


FIG. 4. RELATION OF TOTAL CALCIUM TO THE RESPECTIVE TOTAL PROTEIN CONTENT IN 75 OBSERVATIONS ON MULTIPLE MYELOMA, AS REPORTED IN THE LITERATURE

the respective total protein content fall consistently below the straight line corresponding to Equation I, the divergency tending to be more marked where the total protein content is very low. Decreased  $\text{Ca}^{++}$  may account for this discrepancy. It is known that, associated with hypoproteinemia due to malnutrition, not only the protein-bound but also the  $\text{Ca}^{++}$  concentration may fall—to such low levels that symptoms of tetany may appear. In a broad sense, this condition constitutes a primary disturbance in calcium metabolism in that the intake or absorption of calcium is

abnormal. At any rate, the concentration of  $\text{Ca}^{++}$  cannot be assumed to be within normal limits in malnutritional hypoproteinemia, and we have excluded our cases from the data subjected to analysis.

Cases with a primary disturbance in calcium metabolism, with hyperphosphatemia and with malnutritional hypoproteinemia are excluded, then, on the common ground that in these conditions, the concentration of  $\text{Ca}^{++}$  does not remain within normal limits. It is essential that the  $\text{Ca}^{++}$  concentration remain relatively constant because

in this, as in previous empirical attempts to obtain a quantitative expression of the relation of serum calcium to serum proteins, the effect of varying serum proteins on the *total* calcium content of the serum was considered. Since only part of the total serum calcium is bound to protein, this approach is possible only under conditions in which that fraction of the total calcium which is *not* bound to protein (chiefly  $\text{Ca}^{++}$ ) remains constant. Expressed in terms of Equation I,  $b$  must remain fixed.

This condition is approximated in normal and nephrotic sera, which have been shown by various investigators to maintain a reasonably constant diffusible calcium content at a mean level of about  $5.8 \pm 0.2$  mgm. calcium. This condition obtains also in hyperproteinemia due to lymphogranuloma inguinale (6) and in other diseases included in our series of cases (34).

In primary disturbances in calcium metabolism, in hyperphosphatemia and in severe malnutrition, however, gross fluctuations in the concentration of  $\text{Ca}^{++}$  occur, for reasons already stated. Such fluctuations in the concentration of  $\text{Ca}^{++}$  affect not only the level of ionized calcium but also the amount of calcium bound to protein; since these two fractions tend to maintain an equilibrium at levels which are predictable, as McLean and Hastings (5) have shown, from mass law considerations. Expressed in terms of Equation I,  $m$  being a function of  $b$  (see derivation from the mass law),  $m$  assumes different values as  $b$  varies, and  $m$  is fixed only so long as  $b$  is fixed. Empirical constants for the calcium-binding properties of the serum proteins can be expected to apply, therefore, only under conditions in which the concentration of  $\text{Ca}^{++}$  is the same, i.e., within normal limits.

We have not attempted to estimate protein-bound calcium more directly by ultrafiltration (except in a few instances) or by the McLean and Hastings' (16) frog-heart method for direct estimation of  $\text{Ca}^{++}$ . An attempt was made to calculate  $\text{CaProt.}$  and  $\text{Ca}^{++}$  from the total calcium and total protein (5) or albumin and globulin content (17) of the serum. But this approach was abandoned when it was found that in sera with marked hyperglobulinemia due to lymphogranuloma inguinale, calculated values for  $\text{Ca}^{++}$  were considerably lower than the diffusible calcium (6). In

extreme cases, the calculated  $\text{Ca}^{++}$  concentration was so low as to be within the range of tetany, of which our cases show no signs clinically. As suggested elsewhere (14), constants for  $B$  globulin and  $\text{pK}_{\text{CaProt.}}$  derived from normal serum globulin may not be applicable to globulins occurring in marked hyperglobulinemia.

#### DISCUSSION

Our results are in accord with the view that a direct linear proportionality between total serum calcium and total serum protein, expressed by Equation I, exists in nephrotic and normal sera, from which Equation I was originally derived. When applied to *hyper*proteinemia, however, Equation I leads to gross discrepancies between calculated and observed calcium values (Figures 2 and 3); because such application involves extrapolation on the assumption, apparently incorrect, that the straight line relation found in *hypoproteinemia* holds at elevated serum protein levels.

In Equation I, the common factor  $m$  is used to express the amount of calcium bound per gram of total serum proteins, although the serum proteins are known to be composed of several more or less discrete protein fractions. A common factor may be so employed if the ratio of the respective serum protein fractions to each other remains fixed in diseases affecting the total protein level of the serum; or, should these ratios vary, if the amount of calcium bound per gram of the several serum proteins is approximately the same.

It is now well established that the ratio albumin:total globulin does not remain constant as the total serum protein content rises above or falls below normal limits. *Hypoproteinemia*, as is well known, is due wholly or in large part to decreased serum *albumin*; whereas *hyperproteinemia* appears to be due invariably to increased serum *globulins* (6, 14, 20). Moreover, the several globulins present in serum (as defined by fractional salting out with sodium sulfate) are themselves disproportionately affected when the total globulin content rises. The euglobulin fraction, as defined by Howe's method, almost invariably constitutes most of the globulin increment; often in association with a more or less significant rise in the pseudoglobulin I fraction, as defined by Howe's method. The pseudoglobulin II fraction, however, so far as we could determine (14, 34),

appears to remain within the limits observed in normal serum, despite marked elevations in total globulin content. Thus, while the euglobulin fraction, as defined by Howe's method, often comprises 20 per cent or less of the total globulin in normal serum, it may compose 50 per cent or more of the total globulin in hyperglobulinemia; whereas the pseudoglobulin II fraction, as defined by Howe's method, makes up a third or more of most normal serum globulins, but falls to as low as 15 per cent or less of the total serum globulins in marked hyperglobulinemia.

As to the amount of calcium bound specifically to the several serum protein fractions, nothing definite is known because the results obtained by different methods are conflicting. The prevailing impression, derived chiefly from observations made in various clinical conditions, appears to be in accord with the view expressed by Schmidt and Greenberg (11) that protein-bound calcium is "very largely united to albumin." Similar conclusions were reached by Csapó and Faubl (21), who found less calcium carried down by the globulin fraction precipitated by half-saturated ammonium sulfate than that carried down with the albumin fraction. The results of ultrafiltration experiments with graded membranes have been interpreted (22, 23), in fact, as indicating that all protein-bound calcium is bound solely to albumin. On the other hand, dialysis experiments carried out with normal serum globulin by Loeb (24) clearly indicate that normal serum globulin binds calcium. That normal serum globulin binds a significant amount of calcium was confirmed, among others, by McLean and Hastings (5), who give the following distribution of calcium fractions as typical where the total protein content is 7.0 per cent and the A/G ratio is 1.8:

	Mgm. per 100 grams serum H <sub>2</sub> O
Total Ca.....	11.60
Ca <sup>++</sup> .....	5.16
Ca bound to globulin.....	1.86 (or 0.744 mgm. Ca per gram of globulin)
Ca bound to albumin <sup>2</sup> .....	3.22 (or 0.716 mgm. Ca per gram of albumin)
Ca unaccounted for <sup>2</sup> .....	1.36

<sup>2</sup> McLean and Hastings point out that serum albumin tends to lose some calcium-binding capacity during purification; hence the figure for albumin should be regarded as minimal and that for Ca unaccounted for as maximal.

As will be apparent subsequently, these conflicting results are not mutually exclusive but may well be due, in part, to the heterogeneous and varying composition of the total serum globulin fraction in normal serum and in hyperglobulinemia.

So far as our own results are concerned, if the several serum protein fractions bound approximately the same amount of calcium per gram, Equation I should give satisfactory agreement between observed and calculated calcium values over the range of variation in serum proteins; whereas gross discrepancies occur (Figures 2 and 3). Examination of Figure 3 reveals that the discrepant points are not erratically distributed but, with few exceptions, lie below the straight line corresponding to Equation I; i.e., the discordant sera consistently contain less calcium than that calculated from their total protein content by Equation I. It is further apparent (Table I, Figures 2 and 3) that the discrepant sera are, with few exceptions, those with increased globulin content, irrespective of etiology; i.e., the observed calcium content of most sera with marked hyperglobulinemia is significantly lower than that calculated from their total protein content. This generalization appears to hold whether the increase in serum globulins results in hyperproteinemia (as in lymphogranuloma inguinale) or whether (as in some cases of hepatic cirrhosis) the total protein remains within normal limits because of very low albumin levels. The serum globulin content was within normal limits in our cases with the nephrotic syndrome, in which agreement with Equation I was satisfactory.

That the observed calcium in hyperglobulinemia was lower than predicted was not due to hyperphosphatemia, which was not present in these sera; nor to a decrease in the Ca<sup>++</sup> fraction, which was shown to be within normal limits by ultrafiltration (6) and by the absence of clinical signs of tetany. The discrepancy results because, in hyperglobulinemia, the amount of calcium bound to protein is consistently less than that predicted by Equation I from the total protein content. We infer that the total globulin fraction in hyperglobulinemia binds less calcium than indicated by the several constants proposed in Equation I for *m* (6).

In Figure 5, we have plotted total calcium against the respective total globulin content of

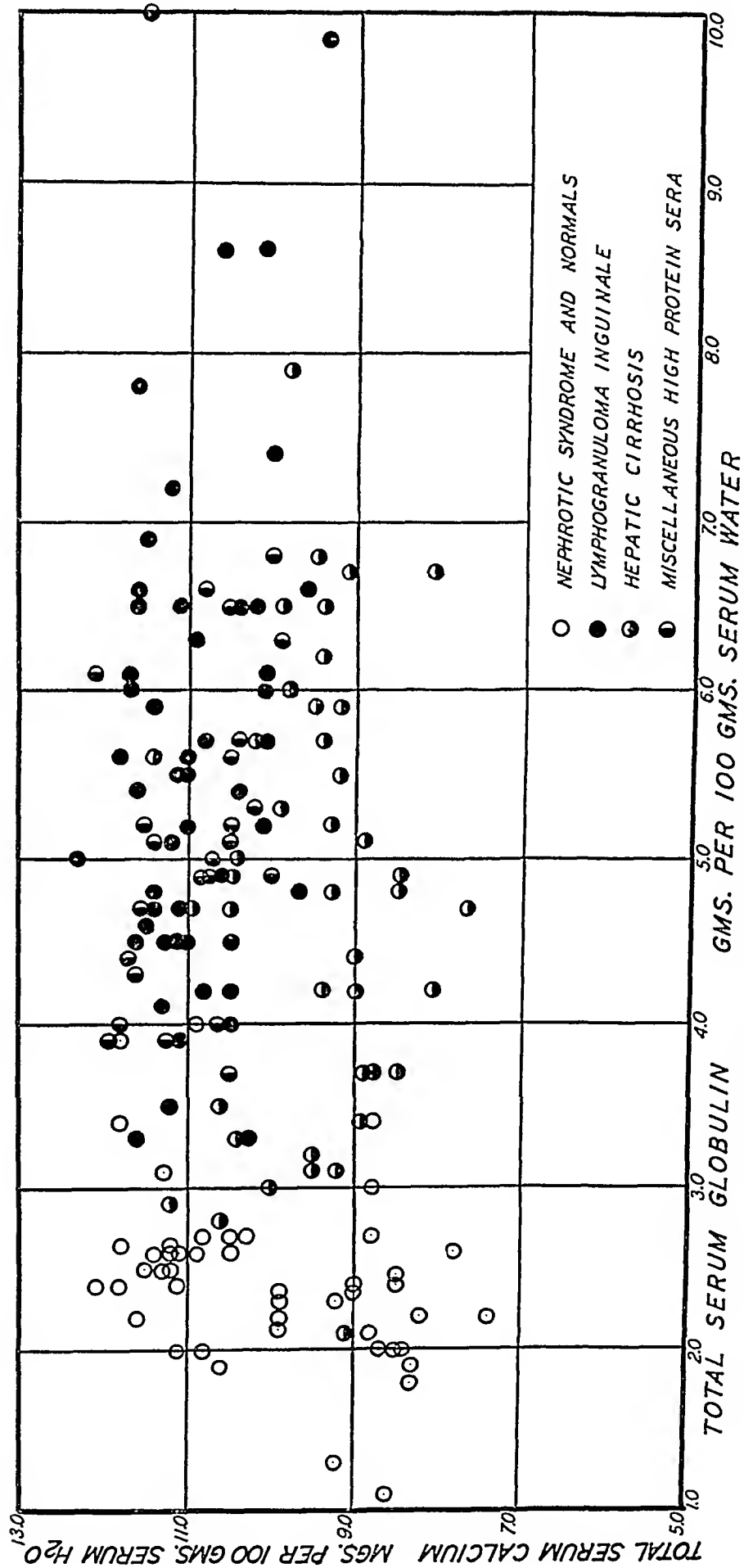


FIG. 5. RELATION OF TOTAL CALCIUM TO TOTAL GLOBULIN IN 164 SERA

Except for hypocalcemia due to low serum albumin, the points lie in a direction approximately parallel to the "x" axis and within the normal range, irrespective of the total globulin level.

our sera. If the protein-bound calcium content of these sera depended chiefly upon their globulin content, the points would fall along a line having a definite slope. We find, on the contrary, that the points fall in a direction approximately parallel to the "x" axis—the correlation between total calcium and total globulin appears to be almost zero. Apart from some low values due to decreased serum albumin content, the total calcium remains within normal limits, irrespective of the total globulin level. The graph suggests that the amount of calcium bound to globulin is either insignificant or small and constant throughout. Figure 5 is not wholly satisfactory, however, because though three variables are involved, only two can be plotted; and more precise statistical analysis necessitates some modification of this initial impression.

In Figure 6, we have plotted total calcium against the respective albumin content of our sera.

The points show a definite trend with a sharp slope. The graph indicates that most of the protein-bound calcium is bound to albumin. That not all the protein-bound calcium is bound to albumin, however, is suggested by the following two observations.

1. It is apparent upon inspection of Figure 6 that the intercept on the "y" (Ca) axis of the trend shown by these points is significantly higher than 5.8 (the mean of observed values for diffusible calcium or  $\text{Ca}^{++}$ ); i.e., a small, constant calcium fraction is bound to protein but not to albumin. Since there appears to be no constant, systematic error in determining either calcium or albumin, and since evidence for a diffusible, non-ionized calcium fraction in significant amount is lacking, we infer that this small, constant calcium fraction is bound to globulin. The amount so bound was estimated more accurately by deriving a linear equation from our normal and nephrotic

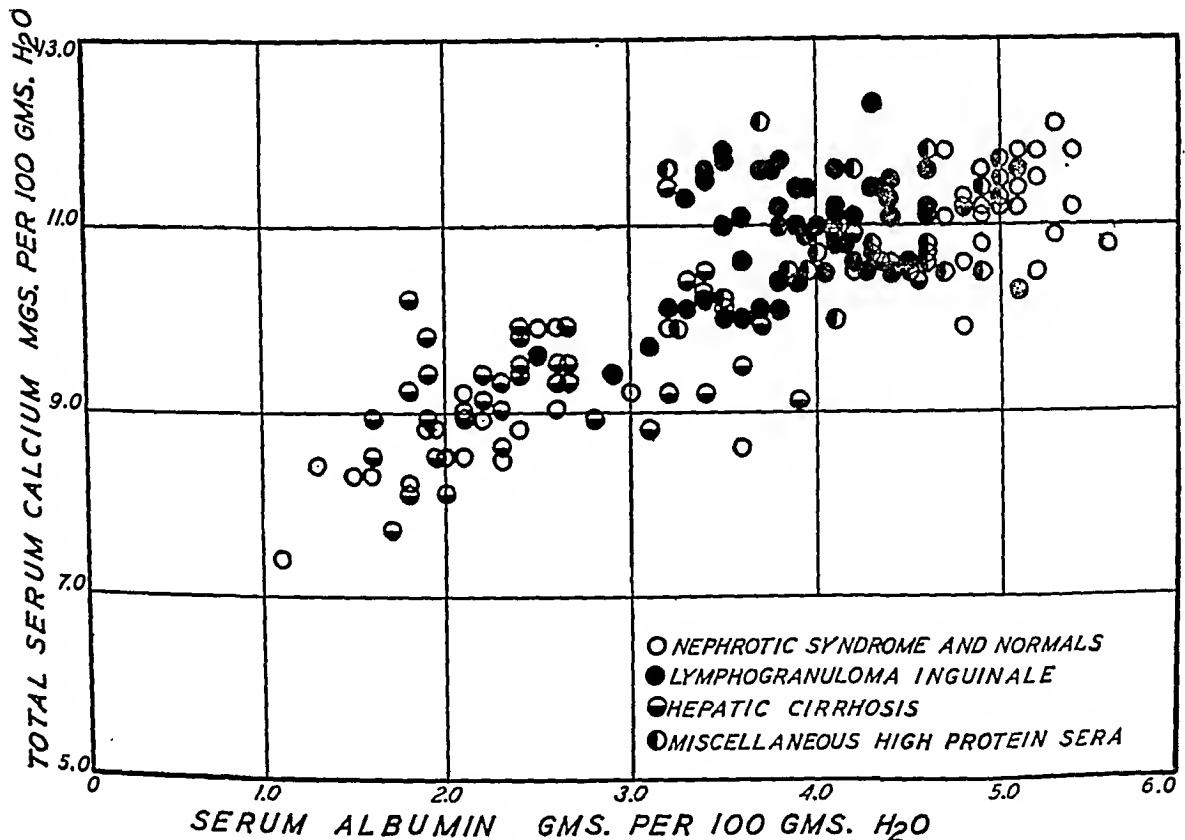


FIG. 6. RELATION OF TOTAL CALCIUM TO ALBUMIN IN 164 SERA

The points show a definite trend with sharp slope, indicating that most of the protein-bound calcium is bound to albumin.

sera by the method of least squares. Calcium is expressed in milligrams, albumin in grams per 100 grams serum water in the equation so obtained:

$$\text{Total Ca} = 0.83 \text{ albumin} + 7.0. \quad \text{III}$$

By subtracting 5.8 from 7.0, the intercept on the "y" axis, we obtain 1.2 as an approximation of the mean amount of calcium bound by this globulin fraction, designated "globulin fraction II."

2. It is further apparent upon inspection of Figure 6 that the majority of points corresponding to lymphogranuloma inguinale and other conditions with marked hyperglobulinemia fall above the general linear trend. Statistical analysis reveals that this deviation becomes appreciable when the total globulin content exceeds approximately 4.0 per cent; and that the deviation is roughly proportional to the degree of hyperglobulinemia. We infer that, in hyperglobulinemia, a small but increasingly significant amount of calcium is bound to the globulin increment responsible for the hyperglobulinemia. This globulin, which we shall refer to as "globulin fraction I," is presumably chiefly euglobulin, partly pseudoglobulin I, as defined by Howe's method. Our calculations indicate that this fraction binds approximately 0.1 to 0.2 mgm. calcium per gram of globulin.

To sum up then, analysis of our data suggests that the total serum calcium is composed of at least four distinct fractions: 1, Calcium bound to and proportional to the albumin fraction; 2, calcium bound to a globulin fraction which remains relatively constant in amount irrespective of the total globulin level; 3, calcium bound in small amount to another globulin fraction, increasing with the total globulin level and becoming significant in marked hyperglobulinemia; 4, calcium not bound to protein, relatively constant because gross fluctuations are excluded by definition. These fractions are represented in the following general regression equation (12):

$$\text{Total Ca} = m_1 \cdot \text{albumin} + m_2 \cdot \text{"globulin fraction II"} + m_3 \cdot \text{"globulin fraction I"} + b. \quad \text{IV}$$

Statistical analysis of our own and published data suggests that these constants have the following limiting values: Where  $b = 5.8 \pm 0.2$  mgm. Ca per 100 grams of serum  $\text{H}_2\text{O}$ ,  $m_1$  is of the order 0.7 to 0.9 mgm. Ca per gram of albumin; the product  $m_2 \cdot \text{"globulin fraction II"}$  is a constant

of the order  $1.0 \pm 0.5$  mgm. Ca per 100 grams of serum  $\text{H}_2\text{O}$ ;  $m_3$  is of the order 0.1 to 0.2 mgm. Ca per gram "globulin fraction I" where "globulin fraction I" is defined arbitrarily as all globulin in excess of 3.0 grams of total globulin.

Values calculated for total calcium by Equation IV, using constants within the limits specified, approximate observed total calcium more closely over the range of variation in serum proteins, than do values calculated by Equation I. This is illustrated in Figure 7 where we have plotted mean ratios, *calculated* total serum calcium: *observed* total serum calcium, against the total protein content.<sup>3</sup> So plotted, a generally valid equation for calculating total calcium will yield points which fall along a straight line at or near 1.00, with zero slope. Equation IV appears to satisfy these criteria. Equation I, on the other hand, yields points diverging progressively upward with increasing total protein (globulin) content.

Figure 7 also illustrates the results obtained with two other general regression equations subjected to statistical analysis (12). In the first of these, the term " $m \cdot \text{total protein}$ " in Equation I is expanded to allow for different calcium-binding properties of the albumin and total globulin fractions:

$$\text{Total Ca} = m_1 \cdot \text{albumin} + m_2 \cdot \text{total globulin} + b. \quad \text{V}$$

In the other, all protein-bound calcium is assumed to be bound to albumin, as suggested by Bendien and Snapper (22):

$$\text{Total Ca} = m \cdot \text{albumin} + b. \quad \text{VI}$$

Both these formulae, like Equation IV, give predicted total calcium values for nephrotic and normal sera quite as satisfactory as those obtained with Equation I (12). And both formulae, like Equation IV, may be derived from general mass

<sup>3</sup> The ratio, total calcium *calculated*: total calcium *observed*, was first calculated for each serum, using Equation IV with the constants indicated in the legend of Figure 7. Then the sera were grouped according to total protein content, expressed in grams per 100 grams of serum  $\text{H}_2\text{O}$ : 3.1 to 6.0 grams, 28 observations; 6.1 to 8.0 grams, 50 observations (20 normal sera, 30 pathological); 8.1 to 10.0 grams, 68 observations; 10.1 or more grams, 18 observations. The mean ratio for the sera in each group was then calculated. The process was repeated for Equations I, V and VI, using the respective constants indicated in the legend of Figure 7.

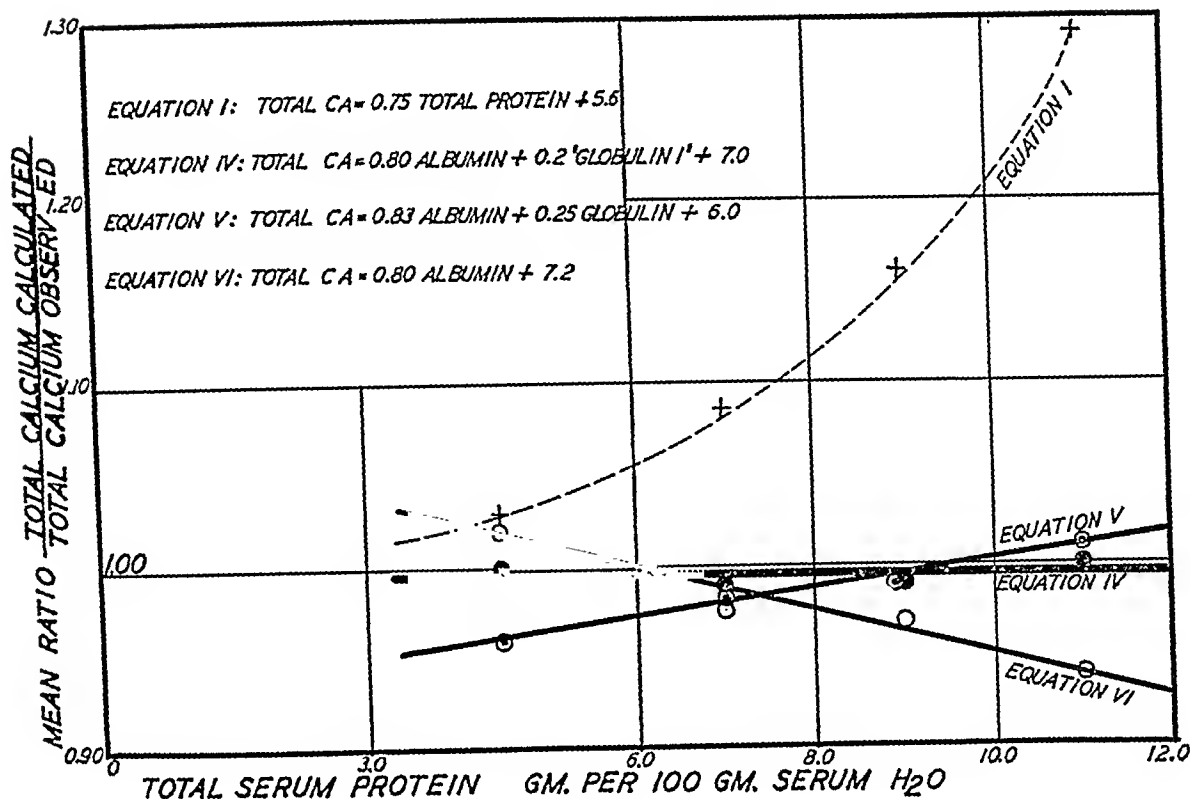


FIG. 7

The mean ratio, *calculated* total calcium: *observed* total calcium, is approximately 1.00 throughout the range of variation in serum proteins when Equation IV is used. Equation I gives increasingly divergent results in hyperglobulinemia. Equation V has a positive slope, Equation VI has a negative slope.

law equations by methods analogous to those used in the derivation of Equation I (see page 903). But as shown in Figure 7, neither Equation V nor Equation VI gives as satisfactory agreement between calculated and observed total calcium in both low and high protein sera as does Equation IV. The points corresponding to Equation V have a definite positive slope because of discrepancies arising from the heterogeneous composition of the total globulin fraction, as suggested elsewhere (6, 12). The points corresponding to Equation VI have a definite negative slope, presumably because the globulin fraction does bind some calcium.<sup>4</sup>

<sup>4</sup>Of course, the position and slope of the lines corresponding to these equations depend, in part, upon the constants employed. For Equation I, means of the published values for  $m$  and  $b$  were used as most representative. For Equations V and VI, we used constants giving the best fit, as determined by the method of least squares. Results obtained by trial of other constants in Equations

"Globulin fraction II," which is relatively constant in amount in all sera irrespective of total globulin content, resembles in this respect the pseudoglobulin II fraction, as determined by Howe's method. Further, the amount of "globulin fraction II" present in serum approximates that of pseudoglobulin II, as we could find no evidence of decreased "globulin fraction II" in sera containing as little as 1.5 grams total globulin. Since the product  $m_2$  "globulin fraction II" is approximately  $1.0 \pm 0.5$  mgm. Ca, this implies

I, V and VI (12) show that the slopes change in degree but not in direction.

The dispersion obtained with Equation IV using the constants indicated in Figure 7 is shown elsewhere by means of a scatter diagram (12). The standard error of estimate is 0.575 mgm. Ca. Sixty-five per cent of the ratios are within  $\pm 5$  per cent of 1.00; in 20 instances the ratio exceeded 1.05, of which 5 exceeded 1.10; in 32 instances the ratio was less than 0.95, of which 7 were less than 0.90.

that "globulin fraction II" binds more calcium per gram than does albumin.

In connection with "globulin fraction I," globulins with isoelectric zones at pH approaching that of serum have been isolated repeatedly from the sera of immunized animals (25, 26, 27, 28). Evidence for the existence of such a globulin in hyperglobulinemia due to kala-azar has been offered by Chopra and Chaudhury (29). We have called attention elsewhere (14) to certain discrepancies in the acid-base equivalence of the blood in hyperglobulinemia (of which the discrepancies in calcium here described are a special case) as indicating the existence of such a globulin fraction.

The inference that the total globulin in normal and pathological sera is composed of varying mixtures of two or more globulin fractions which bind different amounts of calcium may explain a discrepancy already referred to: the apparent conflict between the view that the protein-bound calcium in serum depends chiefly upon the serum *albumin* content and the repeatedly demonstrated binding of considerable amounts of calcium by normal serum globulin. In hyperglobulinemia, the total globulin fraction appears to be composed largely of a globulin which binds little calcium ("globulin fraction I"); whereas a large proportion of normal serum globulin consists of a globulin ("globulin fraction II") which binds appreciable amounts of calcium.

#### SUMMARY

1. The relation of total serum calcium to total serum protein and to the several protein fractions was studied by graphic and statistical analysis of 164 observations on 128 cases. The total protein content of these sera, expressed in grams per 100 grams of serum  $H_2O$ , was: 3.1 to 6.0 grams, 28 observations; 6.1 to 8.0 grams, 50 observations (20 normal, 30 pathological sera); 8.1 to 10.0 grams, 68 observations; 10.1 or more grams, 18 observations. Cases with a primary disturbance in calcium metabolism (including multiple myeloma), with hyperphosphatemia or with malnutritional hypoproteinemia were excluded.

2. It was found that while total calcium is directly proportional to total protein in nephrotic and normal sera, no such relation obtains in hyperproteinemia. Apart from some cases of multiple myeloma, where bone destruction is the prob-

able cause of hypercalcemia, the total serum calcium does not rise in hyperproteinemia but is maintained at normal levels. Equations relating total calcium to total serum protein do not hold when hyperglobulinemia is present. This discrepancy results, apparently, because the globulin increment in hyperglobulinemia binds very little calcium.

3. Analysis of our data suggests that the total serum calcium is composed of at least four fractions: 1, Calcium bound to and proportional to albumin; 2, calcium bound to a globulin fraction which remains relatively constant in amount irrespective of the total globulin level; 3, calcium bound in small amount to another globulin fraction, increasing with the total globulin level but becoming significant only in marked hyperglobulinemia; 4, calcium not bound to protein, relatively constant because cases with gross fluctuations are excluded by definition. (Calcium not bound to protein is itself subdivided into an ionized and a small non-ionized fraction.)

4. A regression equation relating total calcium to albumin and two arbitrary globulin fractions is presented in the following general form:

$$\text{Total Ca} = m_1 \cdot \text{albumin} + m_2 \cdot \text{"globulin fraction II"} + m_3 \cdot \text{"globulin fraction I"} + b.$$

The several constants in this equation appear to have the following limiting values: Where  $b = 5.8 \pm 0.2$  mgm. Ca per 100 grams serum  $H_2O$ ,  $m_1$  is of the order 0.7 to 0.9 mgm. Ca per gram of albumin; the product  $m_2 \cdot \text{"globulin fraction II"}$  is a constant of the order  $1.0 \pm 0.5$  mgm. Ca per 100 grams of serum  $H_2O$ ;  $m_3$  is of the order 0.1 to 0.2 mgm. Ca per gram "globulin fraction I," where "globulin fraction I" is arbitrarily defined as all globulin in excess of 3.0 grams of total globulin.

5. This equation appears to be more generally valid over the range of variation in serum proteins (within the limitations prescribed) than are equations relating total calcium to total protein, to albumin and total globulin, or to albumin alone.

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STUDIES ON SERUM PROTEINS. I. IDENTIFICATION OF A SINGLE SERUM GLOBULIN BY IMMUNOLOGICAL MEANS. ITS DISTRIBUTION IN THE SERA OF NORMAL INDIVIDUALS AND OF PATIENTS WITH CIRRHOSIS OF THE LIVER AND WITH CHRONIC GLOMERULONEPHRITIS<sup>1</sup>

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It has long been known that the globulin fraction of normal human serum contains more than one variety of protein. The separation of this fraction into euglobulin and pseudoglobulin and the further fractionation of pseudoglobulin into pseudoglobulin I and pseudoglobulin II (1) upon the basis of solubility in strong salt solutions confirms this. However, such fractions are still mixtures and can be further divided into subfractions having different chemical and physical properties by dialysis (2) or by partial precipitation with salts (3).

The existence in the globulin fraction of serum of at least two proteins which are antigenically different has been demonstrated by anaphylaxis. Animals sensitized by the injection of the euglobulin or pseudoglobulin fractions are thrown into anaphylactic shock more easily by subsequent injections of the homologous rather than the heterologous fraction (4). Animals immunized with either of the two globulin fractions produce precipitins that react with both fractions. However, quantitative differences in the reactions indicate the presence of two antigens in different proportions in the two fractions and of two corresponding kinds of antibody in the antisera (5).

These observations suggested that if part of the antibody contained in an antiglobulin serum could be absorbed out leaving antibodies to but one of the globulins, such a serum would be a useful guide for the isolation and identification of that globulin. The development of the precipitin reaction as a quantitative method for the estimation of an antigen (6) or hapten (7) makes it possible to use such antisera for the determination of

the actual amount of the varieties of globulin present in human serum or in any of the fractions derived from it.

*I. Preparation of globulin fractions*

Two hundred and ten ml. of human serum, from a patient with hypertension, was separated into euglobulin, pseudoglobulin, and albumin fractions by precipitation at one-third and one-half saturation with ammonium sulfate. The euglobulin fraction was reprecipitated five times by the slow addition of 25 ml. of saturated ammonium sulfate solution to the protein dissolved in 50 ml. of water. The pseudoglobulin was also reprecipitated five times by the addition of 50 ml. of the ammonium sulfate solution to 50 ml. of protein solution.

The euglobulin and pseudoglobulin fractions were freed from sulfate by dialysis against 0.9 per cent sodium chloride solution. Part of the solutions were then fractionated into water soluble and water insoluble fractions by dialysis, first against running tap water and then against distilled water in the ice box.

The water insoluble fractions were dissolved in 0.9 per cent saline. One per cent by volume of a 1 per cent solution of merthiolate<sup>2</sup> was added to each of the fractions, and they were sterilized by filtration through L2 Chamberland filters. Each solution was analyzed by the micro-Kjeldahl technique for total nitrogen and for nonprotein nitrogen.

Five more samples of human serum, four from different patients with hypertension, and one, L2, a mixture of sera from five normal persons were separated into the water soluble and water in-

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<sup>2</sup> Manufactured by Eli Lilly & Co., Indianapolis, Ind.

soluble euglobulin and pseudoglobulin fractions by the method described above.

### *Preparation of antisera*

Alum-precipitated suspensions (8) were prepared by adding 5 ml. of a sterile, 5 per cent solution of crystalline potassium aluminium sulfate to a solution containing 100 mgm. of globulin and then adding 0.4 ml. of 10 per cent NaOH, a quantity sufficient to neutralize the alum solution used. The suspension was diluted to 100 ml. with sterile saline. Rabbits received a course of four intravenous injections a week of this suspension, beginning with  $\frac{1}{2}$  ml. injections the first week, 1 ml. the second and 2 ml. thereafter. After the fourth week, bleedings of from 40 to 50 ml. were made from either the heart or the ear artery, without sacrificing the animal, and after a rest for a week the course of injections was continued. Additional bleedings were then made at intervals of from three to four weeks. After separation of the sera, one per cent by volume of a one per cent solution of merthiolate was added as a preservative, and the sera were sterilized by filtration and stored in the ice box.

### *Reaction of the globulin fractions with rabbit antiglobulin serum Number 389*

A preliminary experiment was made with antiglobulin serum Number 389, prepared by immunizing a rabbit with the water insoluble euglobulin fraction of normal human serum.

Solutions in 0.9 per cent saline of each of the globulin fractions containing about 0.04 mgm. of globulin nitrogen per ml. were prepared. One, two and three ml. samples of each were measured out into 8 ml. Wassermann tubes, the total volume made up to 3 ml. in each case with saline and 1 ml. of antiserum added. After thorough mixing of the contents, the tubes were sealed with "no-air" rubber stoppers, allowed to stand for two hours in an incubator at 37° C., and placed in the ice box overnight. The following morning the tubes were centrifuged for 30 minutes at 2000 r.p.m. in a refrigerated centrifuge at 2 to 4° C. The clear supernatant fluids were carefully poured off and saved for testing. The compact precipitates were resuspended in 1.5 ml. of ice cold saline and re-centrifuged. The supernatant saline solutions were discarded and the precipitates were again

washed with 1.5 ml. of saline. Finally, the precipitates were suspended in saline, dissolved by the addition of a few drops of N sodium hydroxide, transferred to 100 ml. micro-Kjeldahl flasks, and analyzed for nitrogen by the micro-Kjeldahl method.

The results of the analysis are shown in graphic form in Figure 1, where the nitrogen found in the precipitate is plotted against the globulin nitrogen added. Smooth curves of the type shown by Heidelberger and Kendall (6) to be characteristic of the precipitin reaction are drawn through the points for the whole euglobulin fraction and for the two pseudoglobulin fractions. The values found for the two euglobulin subfractions are indicated by circles. A given quantity of the whole euglobulin precipitated more nitrogen from the antiserum than did the same amount of any of the other fractions. Or, expressed in a different way, it required less of the whole euglobulin than of the other fractions to precipitate a given amount of nitrogen. It will be seen from the figure that while it required only 0.042 mgm. of nitrogen in the whole euglobulin to precipitate 0.40 mgm. of nitrogen from the antiserum, it required 0.066 mgm. of the water insoluble pseudoglobulin nitrogen and 0.120 mgm. of water soluble pseudoglobulin nitrogen to precipitate the same amount. This would indicate that these two fractions contained but 65 per cent and 35 per cent as much protein that reacted with the antiserum as did the whole euglobulin fraction. In spite of the fact that the water insoluble euglobulin fraction was used as antigen in the production of the antiserum the figure shows that it was only about 70 per cent as efficient as the whole euglobulin in reacting with the antiserum. These figures do not represent the quantity of any definite antigen in the fractions but refer to the rather vague expression "protein that reacts with the antiserum." The tests upon the supernatant fluids show that more than one kind of antibody is present in this antiserum. In order to use the antiserum for the determination of any particular antigen all of the antibody for other antigens must be removed.

### *Tests upon the supernatant fluids*

Five-tenths ml. portions of the supernatant fluids from the experiment described above were added to 0.1 ml. samples of (a) the antiserum

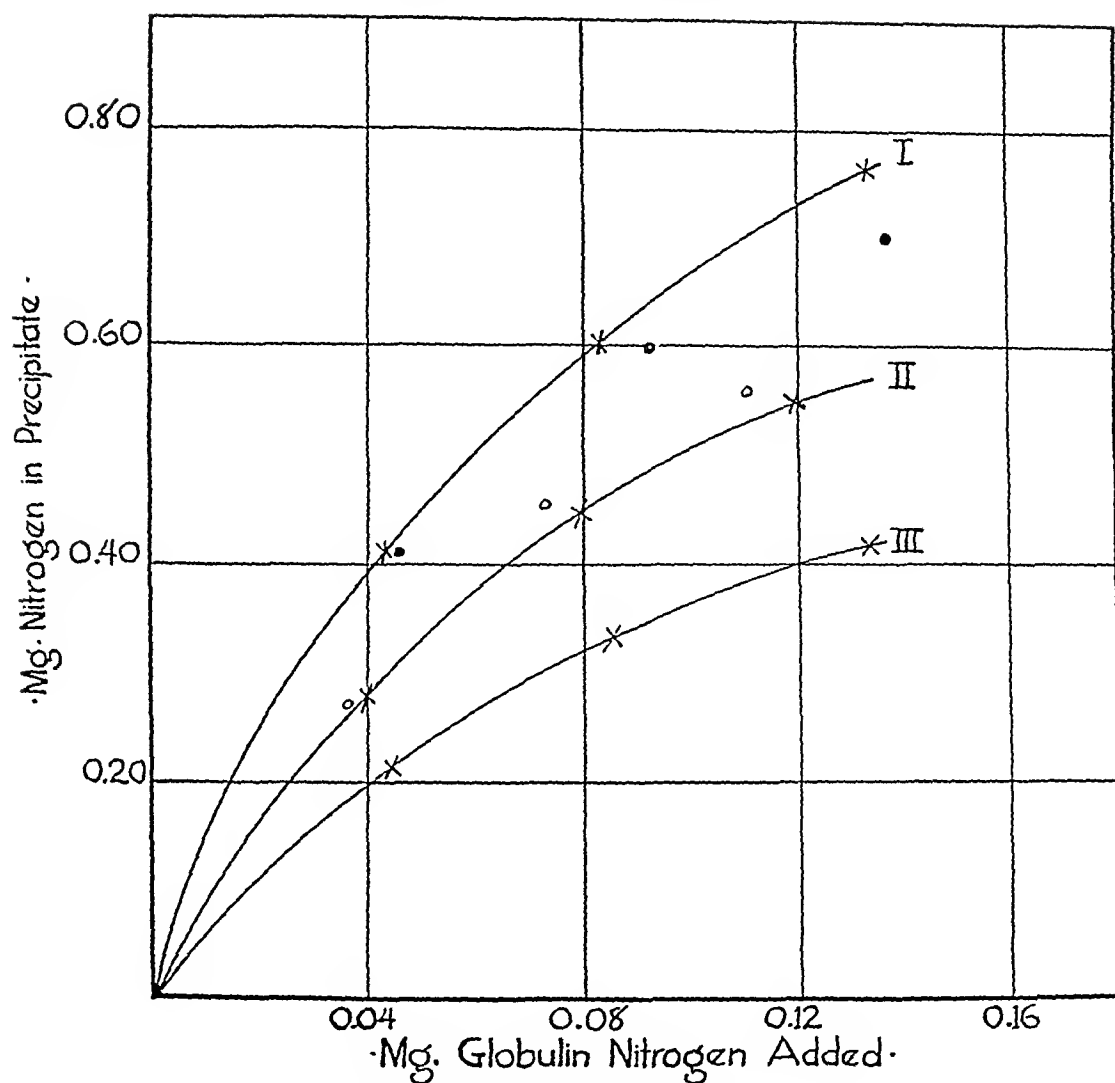


FIG. 1. NITROGEN PRECIPITATED FROM ANTI-EUGLOBULIN RABBIT SERUM NUMBER 389 BY GLOBULIN FRACTIONS

- I Whole euglobulin.
- II Water insoluble pseudoglobulin.
- III Water soluble pseudoglobulin.

- Water soluble euglobulin.
- Water insoluble euglobulin.

and (b) the saline solutions of the different globulin fractions. These mixtures were kept at 37° C. for 2 hours, overnight in the ice box, and were then examined for the presence of a precipitate. The results of these tests are presented in Table I. This experiment demonstrates (a) that the antiserum contained more than one type of *antibody* and (b) that with the exception of the water soluble euglobulin fraction, the globulin fractions were mixtures containing more than one type of *antigen*.

Comparison of the precipitin reaction with a more familiar type of chemical reaction may make the significance of the tests upon the supernatant fluids more apparent. In many of its aspects, the reaction between a protein and its corresponding antibody to form a precipitate is not unlike the more familiar chemical reactions which result in the formation of precipitates. If a solution of silver ions is mixed with one containing chloride ions, silver chloride will be precipitated, and the supernatant fluid will contain only the component

TABLE I

*Nitrogen precipitated from rabbit antiserum Number 389 by globulin fractions*

Fraction	Globulin nitrogen added	Nitrogen in precipitate	Tests upon supernatant fluids					
			Added					
			Anti-serum	Euglobulin	Euglobulin soluble	Euglobulin insoluble	Pseudo-soluble	Pseudo-insoluble
Euglobulin	mgm.	mgm.						
	0.044	0.410	—	+++			++	+++
	0.088	0.598	+	+			+	+
Euglobulin H <sub>2</sub> O soluble	0.132	0.710	+	—			—	—
	0.046	0.410	—		++±	++±		
	0.092	0.598	—		—	±		
Euglobulin H <sub>2</sub> O insoluble	0.137	0.658	+			+		
	0.037	0.270	+		+++	+++		
	0.073	0.454	+		++	++		
Pseudoglobulin H <sub>2</sub> O soluble	0.110	0.562	+		++	++		
	0.044	0.216	+	+++			++	+++
	0.088	0.324	+	+++			++	+++
Pseudoglobulin H <sub>2</sub> O insoluble	0.132	0.418	+	+++			+	+++
	0.040	0.282	+	+++			++	++
	0.080	0.452	+	+++			++	++
	0.120	0.556	+	++			+	+

which had been added in excess. The addition of more silver ions will give a precipitate only if an excess of chloride ions is present, and additional chloride ions will cause precipitation only when there is an excess of silver ions. Both tests will not be positive at the same time. However, if the solutions, instead of containing a single pair of reacting ions, are mixtures containing more than one system, different results may be obtained. Thus, if a solution containing a mixture of silver and barium ions is added to the one containing both chloride and sulfate ions, the supernatant fluid may give a precipitate when tested with either of the two solutions. If the first solution contained 2 equivalents of silver and 3 of barium and the second one 3 equivalents of chloride and 2 of sulfate, the supernatant fluid would contain 1 equivalent each of barium and of chloride and would give a heavy precipitate when added to either the silver-barium or the chloride-sulfate solutions. This behavior in an inorganic system is evidence that it contains more than one pair of reacting ions.

The reaction between antigen and antibody behaves in a similar manner: the formation of a precipitate upon the addition of antiserum indicates the presence of an excess of antigen, whereas a precipitate following the addition of

antigen shows the presence of an excess of antibody. Positive tests for both antigen and antibody are not given by the same supernatant fluid in a system containing a single antigen and its homologous antibody. Positive tests for both constitute definite evidence that the antiserum contains more than one kind of antibody and that the antigen is a mixture of antigenic components.

The results given in Table I definitely show that the antiserum contained more than one type of antibody and that all but one of the globulin fractions were mixtures of antigens. In many cases, precipitates were obtained when the supernatant fluids were tested with either more antiserum or more of the globulin fractions.

The tests upon the supernatant fluids of the series to which the water soluble euglobulin had been added showed an important difference between this fraction and the others. In this series, if the test for excess *antibody* was positive, the test for *antigen* was negative. If the test for excess antigen was positive, the test for antibody was negative. Since the antiserum is known to contain more than one kind of antibody this observation must result from one of two conditions. Either this fraction contains but one antigenic globulin or the different globulins were present in such proportions that all of the different kinds of antibody were removed simultaneously. The second possibility was ruled out by tests with fractions known to be mixtures which showed that those supernatant fluids which failed to react with the water soluble euglobulin still contained an abundance of antibody that would react with the other fractions. The water soluble euglobulin reacts with antiserum as if it were a single antigen.

The antigen contained in the water soluble euglobulin fraction will be referred to, throughout this paper, as *alpha-globulin*. The remainder of the globulin, which may contain more than one antigenic component, will be called *globulin-X*.

The tests upon the supernatant fluids from the series to which the whole euglobulin had been added showed that this fraction contained both *alpha-globulin* and *globulin-X*. The supernatant fluid from the last tube did not react with any of the globulin fractions. All of the antibody for both the *alpha-globulin* and the *globulin-X* had been removed. The difference between the maximum amount of nitrogen precipitated by this frac-

tion and by the water soluble euglobulin which contained only *alpha-globulin* is a measure of the quantity of nitrogen precipitated by *globulin-X*. Figure 1 shows that this amounts to 0.07 mgm. or about 10 per cent of the total quantity. The tests upon the first tube of the whole euglobulin series show that the amount of euglobulin added did not contain enough *globulin-X* both to completely precipitate this small amount of antibody and to leave an excess to react with the test antiserum. The percentage of *globulin-X* in this fraction must be small.

The tests upon the water insoluble euglobulin and the two pseudoglobulin fractions show that even in the first tubes enough *globulin-X* had been added to leave an excess that reacted with the corresponding antibodies in the test portion of antiserum added. The tests upon the last tubes of the series show that the amount of *alpha-globulin* added was insufficient to precipitate all of its antibody. The percentage of *alpha-globulin* in these fractions must be lower than in the whole euglobulin fraction.

Since the antiserum contains a high percentage of antibody that reacts with *alpha-globulin* and only a small amount of antibody that reacts with *globulin-X*, the percentages of "protein reacting with the antiserum" found in the first part of this experiment are approximations of the *alpha-globulin* content of the fractions.

*This experiment shows that the water soluble euglobulin fraction reacts like a pure antigen with antiserum. It shows that the other fractions are mixtures containing more than one antigen and that the antiserum used contained more than one kind of antibody.*

#### *Reaction of alpha-globulin with antiglobulin serum Number 78*

The preceding experiment showed that the water soluble euglobulin fraction reacted with an anti-euglobulin rabbit serum as if it contained a single antigen. In this experiment, the reaction of this fraction is studied with an antiserum prepared by immunizing rabbits with the unfractionated globulin of normal human serum. One ml. of rabbit serum Number 78 was mixed with various amounts of *alpha-globulin* (water soluble euglobulin), and the amount of nitrogen in the resulting precipitate was determined following the

procedure described in the first experiment. The supernatant fluids were tested for the presence of excess antigen and antibody as before. The results are given in Table II. It will be seen from

TABLE II  
*Reaction of the water soluble euglobulin with antiglobulin serum Number 78*

Euglobulin nitrogen added	Nitrogen precipitated		Tests upon supernatant fluids		
	Found	Calculated	Added		
			Anti-serum	H <sub>2</sub> O soluble euglobulin	H <sub>2</sub> O soluble pseudo-globulin
mgm.	mgm.	mgm.			
0.023	0.178	0.178	—	+++	+++
0.046	0.324	0.331	—	+++	+++
0.069	0.464	0.459	—	+++	+++
0.092	0.552	0.561	—	+++	+++
0.115	0.638	0.637	—	+	++
0.137	0.734		—	—	++

the table that no evidence was found that the *alpha-globulin* contained more than one antigen. Although the difference between the successive amounts of *alpha-globulin* added was made small to permit the detection of small amounts of *globulin-X*, no evidence was found for the presence of that protein in this fraction. The tests upon the supernatant fluids show that no antigen was left behind to react with added antiserum at any point in the range studied. In the last tube, in which all of the antibody reacting with *alpha-globulin* had been removed, there still remained antibody reacting with the *globulin-X* contained in the pseudoglobulin fractions. If a second protein is contained in the *alpha-globulin*, it is not a precipitogen, that is, it does not react with antiserum to give a precipitate.

Heidelberger and Kendall (6) have shown that the reaction between a pure antigen and its homologous antibody follows the equation:

$$\text{Mgm. nitrogen precipitated} = AX - BX^2,$$

where *A* and *B* are constants having definite physical significance (see (6)) and *X* represents the amount of antigen nitrogen added. For serum Number 78, this equation is:

$$\text{Mgm. nitrogen precipitated} = 8.3X - 24X^2.$$

The values calculated from this equation are given in Table II where they may be compared with the

corresponding experimental values. The close agreement of the figures is additional evidence that the water soluble euglobulin contains but a single antigen.

*This experiment shows that in its reaction with antiglobulin rabbit serum, the water soluble euglobulin reacts both qualitatively and quantitatively as if it contained a single antigenic component.*

#### *Absorption of antibodies for globulin-X from antiglobulin rabbit serum*

Thirty ml. of antiserum from each of two rabbits, which had been immunized with the whole globulin fraction of normal human serum, were treated with small portions of the water soluble pseudoglobulin fraction, rich in *globulin-X* until tests with unabsorbed antiserum showed the presence of an excess of *globulin-X*. A test portion of the antiserum was treated with an excess of *alpha-globulin*. After centrifuging down the precipitate, the supernatant fluid was tested for the presence of antibody reacting with *globulin-X* by the addition of a dilute solution of one of the pseudoglobulin fractions. A negative test at this point showed that all of the antibody reacting with *globulin-X* had been removed. The serum was filtered through an L2 Chamberland filter and after the addition of 1 ml. of a 1 per cent solution of merthiolate stored in the ice box.

It was found to be more difficult to remove the antibodies for *globulin-X* from antisera obtained by long immunization or from bleedings made after continuing the immunization after the first bleeding. Thus a second bleeding from the two rabbits, described above, still contained antibodies that reacted with *globulin-X* after treatment with the water-soluble pseudoglobulin fraction, as described above. Although the treated sera contained an excess of *globulin-X*, as shown by its reaction with unabsorbed antisera, it still contained antibodies that reacted with the pseudoglobulin fraction after all of the antibodies reacting with *alpha-globulin* had been removed. *This observation is evidence that the globulin-X fraction contains more than one antigen.* In order to remove all of the antibodies for *globulin-X* from such antisera, the absorption had to be continued to a point where a large part of the antibody for the *alpha-globulin* was also removed and the re-

sulting sera were too weak to be used for the quantitative determination of *alpha-globulin*. Therefore, only the first bleedings from the immunized rabbits should be used for the quantitative determination of *alpha-globulin*.

#### *Standardization of the absorbed antiserum*

One ml. of the antiserum was set up with various amounts of *alpha-globulin*, Preparation P-4, in a volume of 4 ml. The tubes were treated exactly as described in the experiments on serum Number 389. All determinations were made in duplicate, except where noted, and calibrated pipettes were used throughout. The quantity of *alpha-globulin* nitrogen used, the amount of nitrogen precipitated, and the tests on the supernatants are given in Table III as well as the values calcu-

TABLE III  
*Nitrogen precipitated from absorbed serum Number 1 by alpha-globulin, P-4*

<i>Alpha-globulin</i> nitrogen	Total nitrogen precipitated		Tests upon supernatant fluids		
	Found	Calculated*	Added		
			Anti-serum	<i>Alpha-globulin</i>	Pseudo-globulin
mgm.	mgm.	mgm.			
0.0174	0.147	0.147	—	+++	+++
0.0347	0.258	0.260	—	++±	++
0.0521	0.329	0.340	—	+	+
0.0694	0.391	0.387	—	±	±
0.0868	0.440		+	—	—
0.1041	0.448†		+	—	—

\*  $N = 9.4x - 55x^2$ .

† Single determination.

lated upon the assumption that the reaction is that of a single antigen-antibody system. It will be noted from the tests upon the supernatants that in no case was the presence of both antigen and antibody noted in the same supernatant, and that no residual antibody was present that reacted with the *globulin-X* contained in the pseudoglobulin fraction.

A chart was prepared by plotting the amount of nitrogen precipitated against the quantity of *alpha-globulin* used and a smooth curve was drawn through the points. It has been shown by previous work (6) that such a curve may be used for the quantitative estimation of an antigen provided certain conditions are controlled. The



amount of antigen in the sample analyzed must not be excessive. Tests upon the supernatant fluid should always be made and should show the presence of an excess of antibody and the absence of antigen. The analysis should be carried out under exactly the same conditions followed in the standardization of the antiserum: i.e., the volume of the antiserum used, the total volume of the reaction mixture, the volume of saline used for washing the precipitate, the length of time the tubes are kept at 37° C. and in the ice box, the temperature at which the tubes are centrifuged, should all be kept constant.

#### *Analysis of the globulin fractions for alpha-globulin*

Solutions of the four globulin fractions, the water-soluble and water-insoluble euglobulins and pseudoglobulins, from a number of different preparations were made up to contain approximately 0.05 mgm. per ml. of nitrogen. Duplicate 1 ml. samples of these solutions were set up with 1 ml. of the absorbed antiglobulin serum Number 1 and the amount of nitrogen precipitated was determined, following exactly the procedure used in standardizing the antiserum. The quantity of *alpha-globulin* equivalent to the nitrogen found was read from the chart.

TABLE IV  
*Alpha-globulin content of globulin fractions*

Preparation	Fraction	Globulin nitrogen added	Nitrogen in precipitate	Equivalent amount of <i>alpha-globulin</i> nitrogen	Per cent of total globulin
P1	Euglobulin H <sub>2</sub> O soluble	mgm.	mgm.	mgm.	
P2-P3 mixture	" " "	0.019	0.320	0.049	100
P5	" " "	0.053	0.332	0.053	100
P6	" " "	0.053	0.332	0.053	100
		0.052	0.324	0.051	98
P1	Euglobulin H <sub>2</sub> O insoluble	0.052	0.244	0.032	62
P3	" " "	0.047	0.226	0.029	62
P5	" " "	0.053	0.224	0.029	55
P6	" " "	0.052	0.224	0.029	56
P1	Pseudoglobulin H <sub>2</sub> O soluble	0.055	0.146	0.017	31
P4	" " "	0.078	0.158	0.023	29
P1	Pseudoglobulin H <sub>2</sub> O insoluble	0.049	0.243	0.032	65
P3	" " "	0.124	0.393	0.070	57
P6	" " "	0.061	0.224	0.029	43

The results of the analyses are given in Table IV. This table showed that, within a small experimental error, the amount of nitrogen precipitated from the antiserum by each of the different preparations of the water soluble euglobulin was

identical with that precipitated by the same quantity of Preparation P-4 which was used to standardize the antiserum. All of the preparations were exactly the same in their ability to react with *alpha-antibody*. This uniformity in the preparations tends to eliminate the possibility that the *alpha-globulin* in this fraction was associated with a second component which did not react with the antiserum. It would not be reasonable to expect preparations made from sera of different individuals to contain a uniform percentage of an inert protein. *The results of the analyses therefore indicate that the water-soluble euglobulin fraction is pure alpha-globulin.*

As indicated by the experiments with the unabsorbed rabbit antiserum, the other fractions are all mixtures containing both *alpha-globulin* and *globulin-X*. The water-soluble euglobulin fractions contained between 55 and 62 per cent of *alpha-globulin*, the water-insoluble pseudoglobulin between 48 and 65 per cent, while the water-soluble pseudoglobulin contained only 30 per cent of this protein. *The variation in the alpha-globulin content of the water-insoluble fractions was small whether the material was prepared from the euglobulin or from the pseudoglobulin fraction. The composition was nearly the same whether the material was prepared from a solution rich in alpha-globulin or one rich in globulin-X.*

#### *Reaction of alpha-globulin with globulin-X*

The constancy of composition of the water-insoluble globulin suggested that it was a chemical compound of the different globulins. If this is true, mixing solutions of the two water-soluble fractions should result in the formation of a precipitate. A solution of the water-soluble euglobulin fraction of Preparation P-4 containing 0.88 mgm. of *alpha-globulin* nitrogen was added to a solution of the water-soluble pseudoglobulin fraction containing 0.77 mgm. of globulin nitrogen, of which 0.54 mgm. was *globulin-X* nitrogen, at 0° C. A heavy precipitate formed immediately. After centrifugation in the cold, the supernatant fluid was analyzed and found to contain 0.42 mgm. of nitrogen, leaving 1.23 mgm. of nitrogen in the precipitate. If the assumption is made that all of the *globulin-X* was precipitated, the insoluble protein contained 56 per cent *alpha-globulin* and 44 per cent *globulin-X*.

The precipitate, which in the cold formed a semi-opaque gelatinous mass, and upon warming to room temperature liquefied to a very viscous transparent fluid, was readily soluble in 0.9 per cent NaCl solution. Dilutions of this solution containing 0.0545 mgm. of nitrogen precipitated 0.230 mgm. of nitrogen from antiserum Number 1, an amount equivalent to 0.030 mgm. of *alpha-globulin* nitrogen. Thus upon analysis, the precipitate was found to contain 55 per cent of *alpha-globulin*, confirming the estimate arrived at above. This value is also in agreement with the values found for the water insoluble euglobulin and pseudoglobulin fractions, and supports the hypothesis that the water insoluble globulin is a compound of *alpha-globulin* and *globulin-X*.

#### *Alpha-globulin content of normal human serum*

Since the absorbed antiserum is specific for *alpha-globulin*, it can be used for the determination of *alpha-globulin* in unfractionated human serum. Thirteen samples of serum from adults in normal health were analyzed for total albumin and globulin by the Howe method (1) and for *alpha-globulin* by the precipitin method. Duplicate determinations of the amount of nitrogen precipitated from 1 ml. of absorbed antiserum Number 1 by the addition of 1 ml. of a 1:100 dilution of each serum were made following the procedure used for the standardization of the antiserum. The amount of *alpha-globulin* nitrogen equivalent to the nitrogen in the precipitate was obtained from the standardization chart. The values for *alpha-globulin* nitrogen were multiplied by the factor 6.25 to obtain values comparable to the albumin and globulin figures from the Howe method. The values for *globulin-X* were obtained by subtracting the *alpha-globulin* figures from the total globulin.

The results given in Table V show that in these thirteen sera the *alpha-globulin* content varied between 1.1 and 2.1 grams per 100 ml. of serum with ten of the values falling between 1.3 and 1.6 grams per 100 ml. These values form from 58 to 78 per cent of the total globulin of the sera. The *globulin-X* content varied between 0.4 and 1.0 with ten of the values lying between 0.6 and 0.8 gram per 100 ml. No correlation was found between any of these values and the values for euglobulin, as determined by the Howe method.

TABLE V  
*Alpha-globulin content of normal human sera*

Person	Date	Sex	Age	Albu- min	Glob- ulin	Eug- lobu- lin	Alpha- glob- ulin	Glob- ulin -X	Alpha- globulin
	1937		years	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	per cent of total globulin
Re....	May 3	F	43	4.3	2.2	0.4	1.5	0.7	63
Ry....	May 3	F	24	5.1	1.8	0.1	1.1	0.1	78
Se....	May 3	M	34	5.1	2.1	0.6	1.6	0.8	67
Ma....	May 3	M	31	5.5	1.6	0.5	1.1	0.5	69
Ka....	May 3	M	33	4.1	2.1	0.1	1.3	0.8	62
Pa....	February 3	M	26	4.9	2.3	0.5	1.1	0.7	61
Do....	June 11	F	21	5.0	2.0	0.3	1.3	0.7	65
Fl....	June 11	M	23	5.0	2.1	0.3	1.4	1.0	53
Ki....	June 11	M	33	4.5	2.7	0.1	2.1	0.6	78
Da....	June 16	F	23	5.1	2.2	0.3	1.4	0.8	64
Me....	June 16	M	23	4.9	2.3	0.4	1.6	0.7	70
Pa....	June 16	M	31	5.3	2.2	0.5	1.4	0.8	64
St....	June 16	M	27	5.0	2.0	0.7	1.9	0.7	73

#### *Alpha-globulin in the serum of patients with alcoholic cirrhosis of the liver and chronic glomerulonephritis*

Two diseases, alcoholic cirrhosis of the liver and chronic glomerulonephritis, were chosen for study because they produce definite changes in the level of serum protein and are marked by certain abnormalities in water metabolism.

The results of the analyses of sera from 13 patients suffering from various stages of alcoholic cirrhosis of the liver are given in Table VI. The ten cases marked with Roman numerals are described by Patek in another paper (9). It will be seen that the high globulin values observed in this disease are due to an increase in the *alpha-globulin*, the *globulin-X* values remaining within the limits found for normal sera. In all of the cases showing extensive liver damage, the *alpha-globulin* formed more than 80 per cent of the total globulin. The cases that showed marked clinical improvement, upon following the treatment described by Patek, showed a decrease in the percentage of *alpha-globulin*.

Table VI also shows the values obtained on seven cases of chronic glomerulonephritis. All of the patients studied in this group were in the nephrotic stage of the disease, showing extensive edema and marked albuminuria. The sixth case, Jo., may have been a case of pure nephrosis. The results show that in six of the cases, although the total globulin was within the normal limits, the *alpha-globulin* was decreased, forming only between 26 and 47 per cent of the total, while the *globulin-X* fraction was increased so that it

TABLE VI  
*Alpha-globulin content of sera from patients with cirrhosis or nephritis*

Patient*	Date	Sex	Age	Albu- min	Glob- ulin	Alpha- glob- ulin	Glob- ulin —X	Alpha- glob- ulin	Comment
	1937		years	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	per cent of total globulin	
ALCOHOLIC CIRRHOSIS OF THE LIVER									
Go. I	April 27	F	45	3.5	3.0	2.4	0.6	80	General improvement. No ascites for 5 months fol-
" "	June 9			3.7	3.0	2.2	0.8	73	lowing 7 months' history of ascites.
Ca. II	May 24	M	63	3.9	3.9	2.7	1.2	69	Continued improvement.
Po. III	April 14	F	49	3.3	2.0	1.6	0.4	80	General improvement. No ascites for 7 months fol-
Har. IV	May 24	M	40	3.7	3.3	2.1	1.2	64	lowing 7 months' history of ascites.
Bo. VI	January 22	M	64	2.9	4.6	4.1	0.5	89	Jaundiced; no ascites. Considered case of alcoholic
" "	February 15			2.6	5.3	4.1	1.2	78	hepatitis.
Br. VII	June 17	F	48	2.9	2.9	2.5	0.4	86	Jaundiced; no ascites. Considered case of alcoholic
Ar. VIII	May 21	F	45	4.3	2.3	1.7	0.6	74	hepatitis.
Tu. IX	May 19	M	52	4.1	4.4	3.7	0.7	84	Ascites of one month duration.
" "	June 11			4.3	4.3	3.4	0.9	79	Ascites present. Bronchopneumonia, causative organ-
Ro. X	April 14	M	49	3.4	3.8	3.1	0.7	82	ism unknown.
" "	June 9			4.0	3.5	2.7	0.8	77	Ascites present. Profound anemia of primary type?
Ha. XI	April 27	F	55	2.1	3.4	2.8	0.6	82	No ascites for 1 month following ascites of unknown
" "	June 9			2.9	3.3	2.7	0.6	82	duration. Anemia.
Has.	May 21	M	66	3.8	3.4	3.1	0.3	91	Ascites for 8 months. Diuresis May 10, 1937.
" "	June 16			3.7	3.0	2.3	0.7	77	No ascites for 1 month.
Ho.	June 5	M	67	1.5	4.7	3.9	0.8	83	No history of ascites. Mild cirrhosis with chronic
La.	June 5	F	38	2.8	2.0	2.0	0.0	100	glomerulonephritis.
CHRONIC GLOMERULAR NEPHRITIS									
Mu.	April 22	M	40	1.9	2.1	0.9	1.2	43	Ascites of 5 months' duration.
Ga.	April 22	M	21	2.2	2.4	0.9	1.5	38	Ascites of 6½ months' duration. Died June 14, 1937.
Wh.	April 14	F	20	2.8	1.9	0.9	1.0	47	Very slight cirrhosis on admission. No ascites.
Ka.	April 14	F	14	3.0	1.6	0.6	1.0	38	Chronic glomerulonephritis?
Hi.	April 12	M	54	2.1	2.8	1.0	1.8	36	Evidence for cirrhosis lacking at this date.
Jo.	May 3	M	15	1.6	1.9	0.5	1.2	26	Ascites 5 months. Died June 29, 1937.
Rh.	April 22	F	46	1.5	3.4	3.0	0.4	88	Possible nephrosis. Died June 5, 1937. Pneumo- coccus peritonitis.
									Generalized edema. Died May 3, 1937.

\* The cases marked with Roman numerals are described by Patek in another paper (9).

formed from 50 to 75 per cent of the total globulin, instead of from 22 to 42 per cent, as found in normal individuals.

The variations in the *alpha-globulin* content of the serum at various stages of these and other diseases are being studied and will be reported in a later paper.

#### SUMMARY

The work reported in this paper confirms the observation of earlier workers that the globulin

fraction of human serum contains at least two proteins that are antigenically different. When rabbits were immunized with the whole globulin or with one of the water-insoluble fractions of the globulin, the resulting antiserum contained precipitating antibodies for at least two different proteins. Quantitative experiments show that most of the antibody in the antisera tested is specific for one of the globulins, antibodies for the other globulin or globulins representing only about 10 per cent of the total amount. It was found pos-

sible to absorb out these antibodies by treating the antiserum with small amounts of a fraction rich in the corresponding globulin and in this way obtain an antiserum that reacted with only one kind of globulin.

This absorbed antiserum was used as a reagent for the quantitative determination of the amount of corresponding protein contained in human serum and in fractions of the serum globulin obtained by precipitation with ammonium sulfate and by dialysis. The information thus obtained led to the isolation of a fraction of the serum globulin that behaved like a pure protein in its reactions with antiserum. This protein, which has been called *alpha-globulin*, was water-soluble and formed from 58 to 78 per cent of the total globulin present in normal human serum. The remainder of the globulin has not been isolated in pure form; the best preparations thus far obtained still contain 30 per cent of *alpha-globulin*. Although evidence has been presented that the remainder of the globulin is itself a mixture of antigens, it has been found convenient to call it *globulin-X*.<sup>3</sup> The purest preparations of *globulin-X* were also water-soluble. When solutions of *alpha-globulin* and *globulin-X* were mixed in the absence of salt, a precipitate was formed which contained 55 per cent of *alpha-globulin*. The composition of this precipitate was very close to that of the water-insoluble globulin obtained when either the euglobulin fraction or the pseudoglobulin fraction is dialyzed free from salts. The constant composition of this protein suggested that it was a compound of the two water-soluble globulins.

#### CONCLUSIONS

1. A fraction of the globulin of normal human serum has been isolated which behaves like a pure protein in its reactions with antiglobulin rabbit serum. This protein has been called *alpha-globulin*.

2. Evidence has been presented that the remainder of the globulin, called for convenience

*globulin-X*, is a mixture containing more than one antigen.

3. Both *alpha-globulin* and *globulin-X* are water soluble. The familiar globulin, insoluble in water but soluble in dilute salt solutions, is formed when these two water soluble fractions are mixed.

4. The water insoluble serum globulin is a compound of *alpha-globulin* and *globulin-X* containing approximately 55 per cent of *alpha-globulin*.

5. Normal human serum contains between 1.1 and 2.1 grams of *alpha-globulin* and between 0.4 and 1.0 gram of *globulin-X* per 100 ml.

6. In a limited series of patients with cirrhosis of the liver or chronic glomerulonephritis with edema, the quantities and proportions of these two proteins are markedly changed from the normal values.

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<sup>3</sup> These names, *alpha-globulin* and *globulin-X*, are preferred by the author to the terms Globulin I and Globulin II suggested by Harris and Eagle (5) since they avoid the possibility of being confused with the terms Pseudoglobulin I and Pseudoglobulin II which refer to certain fractions obtained by precipitation with sodium sulfate.

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# A QUANTITATIVE STUDY OF THE OXIDATION OF GLUCOSE IN NORMAL AND DIABETIC MEN

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There have been two schools of thought as to the cause of diabetes mellitus; one group holding to the lessened oxidation, and another group believing in the overproduction theory of the disease. The information concerning oxidation has been based largely upon repeated determinations of respiratory gaseous exchange at short intervals; the total period being calculated on the assumption that the process was sufficiently uniform to permit the estimation of the total glucose oxidized from a series of samples. The overproduction theory is based on indirect findings, which have not convinced most workers that fat is converted to carbohydrate to any significant extent by the diabetic individual. In addition, there has been much discussion as to whether a diabetic should be fed a diet high in carbohydrate, or one high in fat and low in carbohydrate. The argument in this instance has, in the main, been dependent upon clinical observations. It would seem that the use of a respiration chamber which would permit the continuous measurement of the oxidative processes might clarify these questions.

In this study a respiration chamber (1) for continuous measurement of the respiratory gases has been used. Standard open circuit indirect calorimetry has been employed. Gas analysis has been carried out by the technique of Carpenter (2). The rapidity of mixture of gases has been checked by introducing known quantities of carbon dioxide into the respiration chamber and then removing gas samples at short intervals. It has been found that a complete mixture of gases occurs within three minutes. Alcohol checks (1) have repeatedly given recoveries of better than 99 per cent of the theoretical values.

All of the normal and diabetic subjects have been prepared for three or more days on a simple diet of known composition. Approximately sufficient calories for maintenance were given. The dietary protein has been adequate in all instances.

The diabetics have been given the same quantity of protein as their comparative controls. The quantity of carbohydrate has been varied in the preparatory diet from 21 to 500 grams, and the fat has been adjusted isocalorically. The diabetics were first studied following a long period of control in which their blood sugars were within normal limits. The same diabetics were then investigated after a period of 23 to 35 days of constant hyperglycemia and glycosuria. In some instances, the same diabetics were again studied following a second short period when they were aglycosuric and their fasting blood sugars were kept normal, either by dietary adjustment or by the use of insulin. The amounts of dextrose given at the beginning of the oxidative study have been varied from 0 to 200 grams.

The subjects were placed in the respiration chamber on the evening of the last preparatory day. The following morning, 12 to 14 hours after their last meal, they arose, emptied their bladders, and drank 400 cc. of distilled water, or an equivalent quantity of fluid containing dextrose. They then returned to bed where they quietly reclined for the next four hours. The carbon dioxide and oxygen determinations were begun when the subject had returned to bed. At the end of the four-hour period the subject would again arise and empty the bladder. The carbon dioxide and oxygen determinations were then stopped.

The urine of the diabetics and normal subjects collected during the four-hour oxidative period was routinely tested for ketone bodies with a 10 per cent aqueous solution of ferric chloride. In all instances the test was negative.

The calculations of glucose oxidized were made by the usual standard method (3).

## RESULTS

When three normal male subjects were studied in the fasting state, the amount of carbohydrate

TABLE I

## Normals

*The effect of increasing the carbohydrate of the preparatory diet upon the oxidation of glucose*

Ex- per- iment num- ber	Sub- ject	Diet			Total R. Q.	Total oxy- gen	Nitro- gen of urine	Heat pro- duc- tion	Glu- cose oxi- dized
		Preparation		In cham- ber					
		Cal- ories	Car- bohy- drate	Glu- cose					
			grams	grams		liters	grams	cal- ories	grams
1	C.F.	2000	21	0	.764	66.79	2.60	312	10.6
2	C.F.	2742	53	0	.784	65.47	2.25	309	17.3
3	D.D.	2742	53	0	.790	56.62	2.41	268	20.3
4	C.F.	2750	100	0	.773	64.66	1.84	304	15.3
5	B.D.	2530	100	0	.779	57.97	2.12	273	14.0
6	B.D.	2750	201	0	.792	59.14	2.23	279	17.4
7	B.D.	2687	202	0	.793	60.64	1.99	288	18.7
8	J.M.	3500	500	0	.824	75.44	2.07	360	35.2
9	C.F.	2000	21	25	.777	62.82	2.33	295	14.7
10	C.F.	2742	53	50	.804	70.05	2.25	332	25.6
11	B.D.	1980	50	50	.845	53.06	2.38	253	27.8
12	C.F.	2750	100	50	.833	67.77	2.28	323	33.2
13	B.D.	2750	100	50	.904	56.60	2.43	274	44.9
14	B.D.	2750	201	50	.889	63.09	2.46	305	46.6
15	B.D.	2750	201	50	.870	58.18	1.87	281	39.2
16	C.F.	2750	100	100	.826	71.66	2.05	341	35.1
17	B.D.	2627	101	100	.899	61.37	2.01	298	49.1
18	B.D.	2750	199	100	.921	60.46	2.27	295	53.9
19	B.D.	2627	101	200	.887	62.38	2.22	302	45.5
20	B.D.	2742	204	200	.965	68.05	2.84	333	68.9

TABLE II

## Normals

*The effect of increasing the amount of glucose at the beginning of the period of oxidation when the carbohydrate of the preparation was constant*

Ex- per- iment num- ber	Sub- ject	Diet			Total R. Q.	Total oxy- gen	Nitro- gen of urine	Heat pro- duc- tion	Glu- cose oxi- dized
		Preparation		In cham- ber					
		Cal- ories	Car- bohy- drate	Glu- cose					
			grams	grams		liters	grams	cal- ories	grams
13	B.D.	2750	100	50	.904	56.60	2.43	274	44.9
12	C.F.	2750	100	50	.833	67.77	2.28	323	33.2
17	B.D.	2627	101	100	.899	61.37	2.01	298	49.1
16	C.F.	2750	100	100	.826	71.66	2.05	341	35.1
19	B.D.	2627	101	200	.887	62.38	2.22	302	45.5
14	B.D.	2750	201	50	.889	63.09	2.46	305	46.6
15	B.D.	2750	201	50	.870	58.18	1.87	281	39.2
18	B.D.	2750	199	100	.921	60.46	2.27	295	53.9
20	B.D.	2742	204	200	.965	68.05	2.84	333	68.9

TABLE III

## Normals

*The effect of simultaneously increasing the carbohydrate in the preparatory diet and at the beginning of the oxidation period*

Ex- per- iment num- ber	Sub- ject	Diet			Total R. Q.	Total oxy- gen	Nitro- gen of urine	Heat pro- duc- tion	Glu- cose oxi- dized
		Preparation		In cham- ber					
		Cal- ories	Car- bohy- drate	Glu- cose					
			grams	grams		liters	grams	cal- ories	grams
12	C.F.	2750	100	50	.833	67.77	2.28	323	33.2
13	B.D.	2750	100	50	.904	56.60	2.43	274	44.9
14	B.D.	2750	201	50	.889	63.09	2.46	305	46.6
15	B.D.	2750	201	50	.870	58.18	1.87	281	39.2
16	C.F.	2750	100	100	.826	71.66	2.05	341	35.1
17	B.D.	2627	101	100	.899	61.37	2.01	298	49.1
18	B.D.	2750	199	100	.921	60.46	2.27	295	53.9

TABLE IV

## Diabetic patients

*The effect of increasing the glucose at the beginning of the period of oxidation upon the oxidation of glucose*

Ex- per- iment num- ber	Sub- ject	Diet			Total R. Q.	Total oxy- gen	Nitro- gen of urine	Heat pro- duc- tion	Glu- cose oxi- dized
		Preparation		In cham- ber					
		Cal- ories	Car- bohy- drate	Glu- cose					
			grams	grams		liters	grams	cal- ories	grams
21	M.M.*	2000	21	0	.732	61.69	2.07	287	0.7
22	M.M.	2000	21	25	.757	59.16	1.83	277	8.7
23	M.M.	2000	21	25	.768	59.22	1.67	279	12.2
24	M.M.	2000	21	50	.753	62.93	2.29	294	7.5
25	R.S.†	2700	40	0	.774	68.33	1.34	323	17.5
26	R.S.	2700	40	0	.767	73.61	1.65	347	16.2
27	R.S.	2700	40	50	.780	72.17	2.47	340	17.7
28§	R.S.	2780	130	0	.843	59.53	1.70	286	25.2
29§	R.S.	2780	130	50	.822	63.87	2.39	304	26.5
30§	R.S.	2780	130	100	.791	55.73	2.44	332	21.1
31	R.S.	2780	130	100	.806	57.97	2.25	338	24.4
32	W.H.‡	2600	100	0	.763	56.42	1.46	266	10.3
33	W.H.	2600	100	50	.758	58.37	1.76	274	9.4
34	W.H.	2600	100	100	.801	56.19	2.10	266	18.9
35¶	W.H.	2760	226	0	.803	61.77	3.20	290	19.3
36¶	W.H.	2760	226	50	.820	60.90	3.04	288	23.9
37¶	W.H.	2760	226	100	.818	58.10	3.11	274	21.5

\* M.M. tolerance of 55 grams of available glucose.

† R.S. tolerance of 90 grams of available glucose.

‡ W.H. tolerance of 140 grams of available glucose.

§ Glycosuria and hyperglycemia for 35 days previous to oxidation study.

|| Controlled with insulin with normal fasting blood sugars for 10 days previous to oxidation study.

¶ Glycosuria and hyperglycemia for 23 days previous to oxidation study.



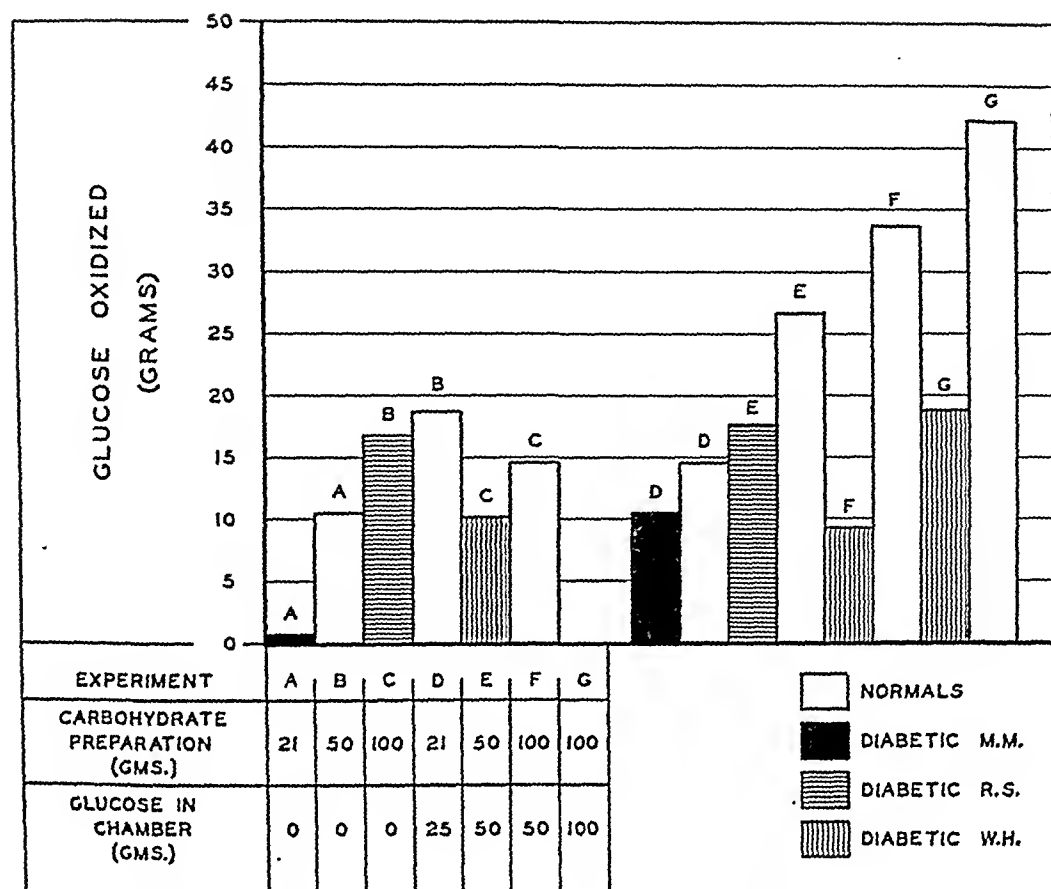


FIG. 1. A COMPARISON OF THE OXIDATION OF GLUCOSE IN DIABETIC SUBJECTS AND NORMAL SUBJECTS

oxidized in the four-hour period became larger as the carbohydrate of the preparatory diet was increased (Table I). This effect was consistently obtained when the carbohydrate in the preparatory diet was varied between 25 and 500 grams. Further, when the glucose was ingested at the beginning of the experimental period in amounts from 50 to 200 grams, even though the carbohydrate preparation had been the same, the oxidation of glucose was increased (Table II). When both the carbohydrate of the preparatory and chamber periods were simultaneously increased, the oxidation of glucose was additive (Table III).

The three male diabetics studied were aged 19, 22 and 26. They were free of any disease other than the diabetes mellitus. All of them had been under observation at the University Hospital for one or more years. The results of the oxidation study in these patients, performed after 14 to 21 days of complete control, are summarized in Table IV. The amount of glucose oxidized in the four

hours was dependent upon the severity of the diabetic's disease. However, the response to increasing the glucose of the chamber period is similar to the response of the normal subjects, but quantitatively reduced. Figure 1 gives a graphic representation of the quantitative difference between the individual diabetic subjects and the normals. In this Figure, *A* and *D* are from one diabetic, *B* and *E* from a second diabetic and *C*, *F* and *G* from a third diabetic. When the same diabetics were studied after a period of 23 to 35 days of constant hyperglycemia and glycosuria, the amount of glucose oxidized in four hours stayed at a constant level (Experiments 28, 29, 30, 35, 36 and 37, Table IV), even though the glucose of the chamber period was increased from 0 to 100 grams. Emphasis should be placed on the inability to increase oxidation of glucose in response to the ingestion of increased amounts of glucose, a marked contrast to the normal controls who oxidized more glucose when they took more

of it. It was also observed that increasing the carbohydrate preparation from two to three times above the tolerance of the diabetics resulted in but slight increase in the amount of glucose oxidized. This slight increase may be attributed to the associated hyperglycemia, which produced a maximum stimulation of the mechanism for the utilization of carbohydrate. Once the maximum stimulation has been reached, further ingestion of glucose can never result in additional oxidation. Experiment 31 (Table IV) further substantiates this view. Diabetic R. S., while taking a diet containing 130 grams of carbohydrate, had a glycosuria that exceeded 60 grams each 24 hours when insulin was not used. This same diabetic was given insulin in amounts to keep him aglycosuric for 10 days. Then food was withheld and insulin discontinued 12 hours before the oxidative study. In this instance 24 grams of glucose were oxidized, an amount similar to the oxidation while on the same diet without insulin. From the above data (Table IV), it is apparent that carbohydrate in excess of the diabetics' oxidatix ability was never beneficial to those diabetics studied nor was it possible to benefit the mechanism that utilizes carbohydrate by the previous use of insulin.

## CONCLUSIONS

The fasting normal subject oxidizes increasing amounts of glucose in response to increasing quantities of carbohydrate in the preparatory diet. He likewise oxidizes more glucose during the four hours in response to increasing ingestion of dextrose at the beginning of the period even though the preparation has been the same. When both sources of carbohydrate are simultaneously increased, the oxidation of glucose is additive. The response of the diabetic is qualitatively similar but quantitatively smaller. The diabetics' ability to oxidize glucose was directly related to the severity of the disease. Carbohydrate, in excess of the ability of these diabetics to oxidize it, was of no benefit.

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